THE THEORY OF EMULSIONS and Their Technical Treatment

By W. CLAYTON, D.Sc., Head of the Technical Development Dept., The Metal Box Co., Ltd., London (*pro tem* at the Ministry of Food). *Fourth Edition.* 103 Illustrations. **42s.**

THE EXAMINATION OF WATERS AND WATER SUPPLIES (Thresh, Beale & Suckling)

Fifth Edition. Revised by E. V. SUCKLING, M.B., B.S., D.P.H. Consulting Analyst to various Water Authorities. **60s.**

THE CHEMICAL ANALYSIS OF FOODS

By H. E. COX, Ph.D., D.Sc., F.I.C., Public Analyst for the Metropolitan Borough of Hampstead. *Second Edition.* 41 Illustrations. **21s.**

PRACTICAL PUBLIC HEALTH PROBLEMS

By Sir W. SAVAGE, B.Sc., M.D., Examiner, Universities of London and Wales, Past President of the Society of the Medical Officers of Health. **10s. 6d.**

ADULTERATION AND ANALYSIS OF FOODS AND DRUGS

By J. F. LIVERSEEGE, F.I.C., Ph.C., formerly Public Analyst to the City of Birmingham. Foreword by the late Rt. HON. NEVILLE CHAMBERLAIN, M.P. **36s.**

J. & A. Churchill Ltd.

CANNED FOODS

AN INTRODUCTION TO THEIR MICROBIOLOGY

By

J. G. BAUMGARTNER

SENIOR BACTERIOLOGIST TO
MESSRS. CROSSE AND BLACKWELL LTD.

Foreword by

W. CLAYTON, D.Sc.

DIRECTOR OF CANNING, THE MINISTRY OF FOOD, LONDON

WITH 15 ILLUSTRATIONS

LONDON

J. & A. CHURCHILL LTD

104 GLOUCESTER PLACE
PORTMAN SQUARE

1943

Printed in Great Britain

FOREWORD

VERY appropriate is the appearance of this monograph relating to the microbiology of food in cans. War gave the original urge to find a practical means of preserving food in hermetically sealed metal containers and successive wars have emphasised the value of canned foods. To-day, not only the armed Forces but the people generally know that canned foods are a munition of war.

Fortunately, in 1939 the food canning industry, especially in Great Britain and the United States of America, was ready for its great test. Canned foods in enormous quantities have been available for fighters and civilians alike. Their special value in chemical warfare is obvious.

Faith in the soundness of canned foods runs high. That faith will rest upon an even stronger basis than past experience when canning technologists all adopt the outlook and methods of control stated by the author of this monograph. Uniformity of laboratory technique and in the interpretation of findings is much to be desired and a *rationale* is here presented.

As the senior bacteriologist in a large modern canning factory, Mr. Baumgartner has unique opportunities. To my personal knowledge over some twelve years he has availed himself to the utmost of those opportunities. I have long admired his careful, critical investigations and I am glad to write this appreciation of his excellent text.

WILLIAM CLAYTON.

PORTMAN COURT,
LONDON

PREFACE

THIS small book had its origin in a series of notes concerning certain microbiological aspects of food canning. The notes were intended for distribution among the members of the technical staff of a canning company and the original material has reached its present form as the result of a suggestion that the information might usefully be given a wider circulation.

The main objects of the book are to present a short account of the principles involved in the processing of canned foods and to indicate the common causes of spoilage and the means by which they can be controlled. The term " canned foods " has been used in its widest sense and embraces glass-packed products which may or may not be wholly preserved by the application of heat. This interpretation seemed desirable in view of the fact that few canners confine their production to heat-preserved food packed in cans. As a result, the destruction of micro-organisms by heat is not the only problem confronting the canning microbiologist or technologist, who needs to be acquainted also with other methods of controlling spoilage organisms. In addition to heat, some of the commoner preserving influences are, therefore, briefly described. For the guidance of those proposing to carry out the bacteriological examination of canned foods, the necessary procedure is indicated. It has been assumed that persons undertaking such work will already be familiar with elementary bacteriological technique and methods and no attempt has been made to include details of these.

Although principally designed for chemists and technologists engaged in the industry, it is hoped that this outline may also be of some value to others who although not

directly concerned in canning, have an interest in the keeping qualities and soundness of canned foods. With this in mind, a short account of the operations involved in canning is included.

It will be apparent to anyone familiar with the literature dealing with canning microbiology that full use has been made of numerous publications, especially those by American authors. For assistance in the preparation of the book, I am greatly indebted to many persons. In particular, I wish to record my sincere thanks to Mr. S. Back, B.Sc., F.I.C., Chief Chemist to Crosse and Blackwell Ltd., for invaluable advice and criticism. Thanks are also tendered to Mr. D. W. Bouchier, B.Sc., A.I.C., for a great deal of assistance and innumerable suggestions. Acknowledgment is also due to : Mr. E. F. Bennett, who very kindly prepared the line drawings with which the book is illustrated ; Messrs. T. E. Bashford, J. G. Huntley, B.A., and C. E. Kessell, who have freely given advice and assistance on several matters ; Mr. T. C. Sutcliffe for the loan of the block for Fig. 4 and to the Editor of "Food" for a similar service in respect of Fig. 6.

Finally, I am deeply conscious of the honour conferred on this book by the inclusion of a foreword by Dr. William Clayton. For this and many other kindnesses, I offer him my deepest thanks.

J. G. B.

CONTENTS

MICROBIOLOGY OF CANNED FOODS

CHAPTER I

MICRO-ORGANISMS

THE bacteria, moulds and yeasts, collectively termed micro-organisms, are usually classified as *Fungi* or *Mycetes*, a sub-family of the *Thallophyta* or flowerless plants which are not differentiated into roots, leaves and stems. The *Fungi* may be further sub-divided into *Schizomycetes* (Bacteria), *Eumycetes* (True Fungi, which include the moulds and yeasts) and *Myxomycetes* (slime moulds). Only the Bacteria and True Fungi are significant in canning microbiology.

Bacteria

Morphology. Bacteria are minute unicellular plants which do not contain chlorophyll. The cells are of many shapes, the three basic forms being spheroidal, cylindrical or rod-like and spiral. Bacterial multiplication takes place by division of the cells, the spheroidal forms—which are termed cocci—dividing in any plane whilst the rod-shaped types divide transversely. With many types of bacteria the cells remain attached to each other following division and groups of cells are formed, the arrangement of which depends on the planes through which division occurs. This grouping of the cells is very important in the classification of bacteria. With the coccal forms several groupings are observed. Cocci which divide in one plane only form pairs of cells known as diplococci or chains termed streptococci. Those dividing through any plane form irregular groups or clusters known as staphylococci. Division in two planes at right angles to each other results in the formation of a group of four cocci as in the tetracocci; division in three planes forms cubes of

eight cells, the name Sarcina being given to groups of this type. With the rod-shaped organisms, to which the general term bacillus is applied, transverse division limits the possible arrangements to pairs or chains of cells.

Size. The extreme smallness of bacteria is indicated by the fact that the unit of measurement is the micron (μ), which is equivalent to one-thousandth of a millimetre. Each species exhibits more or less characteristic dimensions although irregularity may occur due to changes in environmental conditions and other causes. Between the different types there is a very wide variation in size, some species being several times larger than others. For many spheroidal forms the diameter lies between 0·75 and 2μ. A medium-sized rod has a width of between 0·5 and 1μ and a length of about 3μ. Some rods develop filamentous forms which may measure 100μ or more in length.

Structure. The detailed structure of the bacterial cell has occupied the attention of numerous investigators and much conflicting evidence has resulted, chiefly due to differences in the methods adopted. The opposing views that have arisen are cited in a comprehensive review of the literature on bacterial cytology by Lewis (1941). The application of the electron microscope, however, seems likely to lead to important advances in the study of bacterial structure. From what is known at the present time it seems that the bacterial cell comprises an outer cell wall within which is a semi-permeable membrane enclosing the cytoplasm. Various granular inclusion bodies are often present in the cytoplasm but there is no true nucleus such as that found in the cells of higher plants.

Flagella. Many species of rod-shaped bacteria and a few cocci are capable of movement in liquids by means of flagella which appear as fine, whip-like appendages continuous with the cell wall. Flagella are not normally visible unless stained by special processes. Evidence has been produced (Polevitsky, 1941) that flagella are thin-

walled hollow tubes. Rhythmic contraction of the cytoplasm causes changes in pressure within the lumina of the flagella which results in their movement and consequent propulsion of the cell. The location and number of flagella on the cells are regarded as characteristic for a given species but as a routine it is sufficient for identification purposes to determine motility.

Spores. In certain bacteria spores or endospores are formed. The vitality of the actively growing or vegetative cell is transferred to the spore of which only one is formed by each cell in the great majority of species. Spore formation is not a reproductive process but is a means of preserving the species since spores are capable of withstanding adverse conditions such as would be intolerable to the vegetative cell. Spores, which appear to arise from a condensation of cell material, become surrounded by a thick membrane or spore wall. It has been suggested that the high resistance to heat of bacterial spores is due to the insulating qualities of the thick spore-wall, a view which is not generally accepted. Microscopically, spores appear in unstained preparations as round or oval bodies which are much more refractile than the remainder of the cell. When the spore is fully formed, the vegetative part of the cell frequently disappears, leaving the spore as a thick walled, round or oval body. Spores are usually produced only by certain of the rod-shaped species but spore formation has been reported for some species of cocci. The position of the spore in the cell and its size and shape are usually characteristic for each species but they are not absolutely constant (Topley and Wilson, 1936). Spore formation is influenced by many factors. The *p*H range within which sporulation occurs is usually narrower than that which permits growth and the presence of fermentable carbohydrate inhibits spore formation, due to the production of acid in the medium. The temperature range which permits spore-formation is also frequently less than that within

which growth can occur. The earlier view that spore formation arises as a result of unfavourable environment cannot be substantiated. According to Daranyi (1930) spore formation is brought about by colloidal dehydration in the cell as it ages. Kaplan and Williams (1941) believe that spore formation by *Clostridium sporogenes* is a normal function in the life cycle of the organism.

Capsules. Some species of bacteria are distinguished by an ability to surround themselves with sheaths or capsules, mainly of a carbohydrate nature. Lewis (1941) concludes that the capsular material is probably a secretion product of the cell. Special technique is necessary to stain capsules and in ordinary stained preparations encapsulated cells appear to be surrounded by a clear zone or halo. Capsule formation is influenced by environmental conditions. In some species capsular material has specific immunological properties.

Chemical Composition of Bacteria. The chemical constituents of bacterial cells must obviously be related to the environment in which they were produced and this no doubt accounts for some of the widely discrepant analyses recorded in the literature. Bacteria contain a high proportion of water, for most species the water content lying between 75–85 per cent. Anderson (1938) quotes figures of 73·3 per cent. for *Escherichia coli* and 98·3 per cent. for *Acetobacter aceti*. With regard to the protein content, the total nitrogen figures recorded vary considerably but Topley and Wilson (1936) state that the usual range is from 8 to 15 per cent. of the dry weight. Of this at least 80 per cent. appears to be coagulable. The chief constituent of bacterial ash is phosphorus, together with sodium, potassium, magnesium, calcium, silicon, sulphur and chlorine. Recorded figures for total ash range from 2 to 30 per cent.

Nutrition. Before bacteria can proliferate they need to be supplied with materials from which they can derive energy and synthesise the protein, fat, carbohydrate and

other constituents of which the bacterial cell is composed. In a monograph reviewing the subject of bacterial nutrition Knight (1936) states : " The nutrients must supply :—

" (*a*) Material which is ultimately transformed into living multiplying organisms.

(*b*) The necessary energy which is required for this transformation and for other less energetically important purposes such as the maintenance of the new organisms, and the performance of external work (motile organisms) etc."

Bacteria can be separated into two broad groups on the basis of the form in which the necessary nutrients can be utilised. Those which can synthesise their protoplasm from simple inorganic compounds are termed autotrophic. These organisms assimilate carbon as carbon dioxide and nitrogen from ammonia, nitrate or nitrite. They obtain their energy for growth from oxidation reactions (chemosynthetic) or utilise the radiant energy of sunlight (photosynthetic). The autotrophs, which include the nitrifying bacteria important in soil microbiology, have no significance in the spoilage of foodstuffs.

The heterotrophic organisms are those for which organic materials are necessary as a source of carbon and energy. This group includes the pathogenic or disease-producing organisms and the saprophytic types which are responsible for the spoilage of foods. The simpler heterotrophs can assimilate nitrogen from inorganic sources but the more exacting types require amino-acids of varying complexity and in some cases specific amino-acids such as tryptophane are essential. In the usual laboratory media used for the cultivation of bacteria, peptone and meat-extract broths serve as a source of carbon and nitrogen.

With regard to the mineral requirements of bacteria, Knight points out that the inorganic compounds may perform several functions in nutrient media. Their effects may be physical, *e.g.*, as buffer salts to oppose *p*H changes in the medium, and as salts for controlling osmotic and

permeability effects. They may be required as nutrient salts for use in synthesising protoplasm or they may be utilised for catalytic or enzymic purposes. To satisfy the mineral requirements of bacteria, K, Na, Ca, Mg, Fe, phosphate, sulphate, carbonate and chloride ions appear to be necessary. In the preparation of ordinary nutrient media these ions normally occur in sufficient concentration to obviate the necessity for adding them to the medium.

For some organisms specific growth-promoting substances are essential; their nature is not fully understood in all cases. These substances, which are often referred to as bacterial vitamins, are active in very low concentrations. The essential growth factors for some organisms can be synthesised by other types and Knight has suggested that these growth factors may be of wider importance in the growth of bacteria than is generally realised. If an organism is capable of synthesising the factor for itself the need for it is not apparent; the necessity for growth factors only becomes obvious for those organisms which have lost the ability to synthesise them. The subject of accessory growth factors for bacteria has been reviewed by Koser and Saunders (1938).

Bacteria absorb their nutrients through the permeable cell wall. If the material available is in a non-assimilable form it must first be broken down into simpler compounds which can pass into the cell. This conversion is accomplished by enzymes elaborated by the bacteria. After passing into the cell the material undergoes further enzymic changes and may be directly utilised for building cellular substance or broken down into yet more simple compounds. Bacteria vary widely in their enzymic activity, some species for example readily utilising a wide range of carbohydrates while others are inactive or attack only a few. Other species are particularly active against proteins, and fats may be decomposed by certain types. The end-products resulting from these decompositions also show great variation with

the different species. Hydrogen, carbon dioxide, alcohol, lactic, and other acids are some of the products which may result from the decomposition of sugars (usually referred to as fermentation). The splitting of proteins, termed putrefaction, results in the formation of amino acids which may eventually be decomposed to give ammonia and carbon dioxide. Other products of protein decomposition include hydrogen sulphide, indol and other foul-smelling compounds. Pure proteins are not attacked by bacteria unless there is some other nitrogen source such as amino-acid present to permit growth and the formation of proteolytic enzymes. Detailed information concerning bacterial nutrition and biochemical activity is given in Knight's monograph and Anderson's " Introduction to Bacteriological Chemistry."

Oxygen Relationships. In relation to the influence which molecular oxygen of the atmosphere has on their development, bacteria are usually classified as follows :—

(1) Obligate aerobes—requiring molecular oxygen for growth.

(2) Obligate anaerobes—unable to develop in the presence of molecular oxygen.

(3) Facultative anaerobes—growing in the absence or presence of molecular oxygen.

(4) Micro-aerophiles—growing under an oxygen tension less than that in air.

For details of the various theories concerning the *rôle* of molecular oxygen in bacterial respiration, Topley and Wilson (1936) and the monograph by Hewitt (1936) should be consulted. As far as the anaerobic bacteria are concerned it is established that they are unable to develop in media with oxidation-reduction potentials (Eh) above certain limits, the inhibitory effect of oxygen on this class of organism being due to its action in raising the Eh of the media above the minimal limit. The growth of anaerobic bacteria, therefore, requires that molecular oxygen be excluded from the medium or the medium be so compounded that its Eh is

poised at a suitably low level. In the laboratory cultivation of anaerobes, both of these procedures are used. Cultures in the ordinary type of nutrient broth or agar are stored in an inert atmosphere of hydrogen or nitrogen or, in the case of liquid cultures, the surface is sealed with paraffin oil or wax. Such liquid media must be boiled and rapidly cooled before inoculation, in order to remove dissolved oxygen. In the second method, strongly reducing conditions are maintained in the medium by the addition of such substances as glutathione, cysteine, ascorbic acid or cooked meat which are effective even when the medium is exposed to air; this method is applicable only to liquid media and for surface growth on solid media storage in an oxygen-free atmosphere is essential.

Bacterial Toxins. The pathogenic bacteria are characterised by the production of substances causing diseases or illness as a result of their injurious effect on the body tissues. These poisonous substances, known as toxins, are antigenic, that is they stimulate the body tissues in the production of various specific neutralising substances which are collectively termed antibodies.

The ability to stimulate antibody production is not confined to living bacteria. Dead bacteria are antigenic as are all non-living proteins of colloidal dimensions provided they are foreign to the animal or host into which they are injected. Most toxins are proteins or have protein components, but non-protein materials such as poly-saccharide-phosphatide complexes which are toxic and antigenic have been isolated from bacteria.

Two main types of bacterial toxins are generally recognised, but it should be noted that absolute distinction is not always possible because of the fact that some toxins exhibit properties common to both types. The classification is based on the following characteristics :—

1. *Exotoxins.* Toxins of this class are extracellular, that is, they diffuse freely into the medium in which the bacteria

are growing and are, therefore, easily separated from the cells by filtration. Exotoxins, which are active in very small amounts, stimulate the formation of the antitoxin type of antibody which specifically neutralises exotoxin and renders it harmless. The symptoms or lesions produced in the body by exotoxins are highly characteristic so that it is usually possible to recognise an exotoxin by the effects it produces. Toxins of this type are generally heat-labile, being inactivated at 60 deg. C. (140 deg. F.) in an hour. Typical exotoxins are those produced by the botulism, tetanus and diphtheria organisms.

2. *Endotoxins.* This type of toxin appears to be an integral part of the cell and cannot be separated from the organisms by filtration. The symptoms produced in the body by endotoxins are less characteristic than those caused by exotoxins and can only be produced by the actual cells or cell extracts. Endotoxins do not usually stimulate antitoxin formation, the antibodies produced being of the anti-bacterial type such as the bacteriolysins which directly destroy bacteria or the agglutinins which immobilise them and cause them to aggregate into clumps. In comparison with typical exotoxins, endotoxins are relatively heat-stable. Most of the pathogenic bacteria produce toxin of the endotoxin class.

The reactions which occur between antibodies and antigens show a relatively high degree of specificity and since many of these reactions are readily demonstrated *in vitro* they are of a great value in the identification of various antigens such as bacteria and proteins. For such purposes the serum of an animal or person is used in which antibodies have developed as the result of injection or infection with the antigen substance. In particular, the agglutination test is widely used for identifying and separating species of bacteria which are morphologically and biochemically indistinguishable. The subject of bacterial toxins and immunity reactions is treated in great detail by Topley and Wilson (1936).

Multiplication and Growth. The mode of reproduction in bacteria is discussed by Lewis (*loc. cit.*) who concludes that there is little evidence for believing that sexual reproduction occurs. Multiplication takes place through the process of fission or division of the cell. Recent work by Knaysi (1941) indicates that first there is retraction of the cytoplasm followed by the formation of two separate transverse walls which bisect the cell. The rate at which the process occurs varies with different organisms and is affected by numerous factors such as the age of the culture, temperature and available nutrients. The rate is maximal at the optimum temperature.

The growth of a bacterial population shows four distinct phases :—

1. *Lag Phase.* During the first phase which may last some hours, although there is little or no actual increase in numbers, growth or enlargement occurs in some of the cells, and there is an increase in respiratory and other processes. When organisms are in the lag phase they show an increased susceptibility to the effects of heat and toxic chemical agents. The length of the lag phase is affected by many factors. It may be decreased by increasing the initial number of cells in the inoculum. Organisms taken from a culture in the logarithmic phase show no lag in the fresh culture and continue to increase at a logarithmic rate. Unfavourable conditions of environment have the effect of prolonging the lag phase. According to Walker (1932) the lag phase may be due largely to the necessity for the organisms to produce carbon dioxide to a concentration essential for growth. Further information concerning the early phase of bacterial growth is available in the review of the subject by Winslow and Walker (1939).

2. *Logarithmic Phase.* This is the period of most rapid growth. The organisms increase in geometrical progression and a straight line graph results from plotting the logarithms of the numbers of cells against time. Under the most favour-

able conditions the more rapidly growing organisms undergo division in about 20 minutes. The length of the logarithmic phase varies with different species, temperature, etc. At the end of the logarithmic increase, the cells recover their normal resistance to adverse physical and chemical agencies.

3. *Stationary Phase.* When, at the conclusion of the logarithmic phase, the number of organisms reaches a maximum, the rate of division gradually becomes slower and for a period is just about sufficient to balance the number of cells dying, thus keeping the number of living cells stationary.

4. *Phase of Decline.* During this phase the total number of viable cells decreases. The rate of decrease varies with the organism and the population may die completely within a few days or the decrease may extend over a period of months. The presence of fermentable sugar in the medium causes acceleration of the decline due to the toxic effect of the acid produced during earlier stages of growth.

The factors which limit the size of a bacterial population are not precisely known. The cessation of growth has been ascribed to various causes, including the toxic effects of metabolic waste products, the formation of specific growth-inhibiting compounds, physical over-crowding, and the exhaustion of food and energy materials. The effect of physical over-crowding and the formation of specific growth inhibiting substances is discounted by Cleary, Beard and Clifton (1935) who believe that growth eventually ceases because of the lack of suitable material for nutritional and energy purposes, the weakened cells dying as a result of the effect of toxic metabolic waste products.

Temperature Range for Growth. For all bacteria there is a minimum and a maximum temperature at which development occurs. Between these points there is an optimum temperature—this usually being defined as that temperature at which cell reproduction is most rapid. The optimum temperature for cell reproduction does not necessarily coincide with that for other processes such as rate of fer-

mentation or for the production of the largest total cell population at the end of the phase of decline. Dorn and Rahn (1939) have shown that *Streptococcus lactis* in milk gave the most rapid growth at 34 deg. C. (93·2 deg. F.); the largest cell population at 25–30 deg. C. (77–86 deg. F); the most rapid fermentation at 40 deg. C. (104 deg. F.) and the highest final acidity at 30 deg. C. (86 deg. F.). Hess (1934) has reported that when total cell population is used as a criterion the optimum temperature for *Pseudomonas fluorescens* and other organisms is 5 deg. C. (41 deg. F.).

With respect to their growth temperature requirements micro-organisms may be separated into three broad groups.

Those with an optimum temperature between 25–40 deg. C. (77–104 deg. F.) are termed mesophilic; members of this group do not as a rule develop below 5 deg. C. (41 deg. F.) and their maximum growth temperature is usually about 40–45 deg. C. (104–113 deg. F.) although certain thermotolerant spore-forming mesophiles will grow at 55 deg. C. (131 deg. F.). The mesophilic group includes all the pathogenic bacteria.

The second group, often referred to as psychrophiles, develop best at a temperature of 20 deg. C. (68 deg. F.) or below. These types develop readily at 0 deg. C. (32 deg. F.) and growth occurs at temperatures down to about —5 deg. C. (23 deg. F.) on unfrozen media (Haines 1934).

The thermophilic group have an optimum temperature at 55 deg. C. (131 deg. F.) or higher, with a minimum temperature of 20–25 deg. C. (68–77 deg. F.) and a maximum of 75–80 deg. C. (167–176 deg. F.). These temperatures should not, however, be regarded as definite limits since there is no sharp demarcation between the groups.

Temperatures outside the range for growth have a lethal or inhibitory action on bacteria. The effects of temperature, together with other controlling influences, are dealt with later.

Identification. The morphological features of the

individual cells are only sufficient to permit of a primary grouping of bacteria and in order to distinguish the different types additional criteria have to be employed. Classification is, therefore, based on the determination of a variety of characteristics briefly indicated below :—

Morphology. Size, shape and arrangement of the cells. Motility (number and arrangement of flagella). Spores (size, shape and position). Capsule formation.

Staining Reaction. Gram's method. By means of the Gram staining method all bacteria can be placed into one of two groups—Gram-positive or Gram-negative. The essential feature of this important staining procedure is that after staining with one of certain of the aniline dyes and treatment with iodine solution some organisms (Gram-negative) can be decolorised by alcohol while others (Gram-positive) retain the dye.

Cultural Characteristics. Type of growth produced in solid and liquid culture media. The size, shape, structure and pigmentation of discrete cell-masses or colonies on solid media are noted. In liquid media, pellicle formation, degree of turbidity and type of sediment (*e.g.*, granular, viscid, etc.) are important. Other cultural tests such as growth in gelatin stab and agar streak cultures are also used.

Oxygen Requirements. Anaerobic, aerobic, facultative, micro-aerophilic.

Temperature Requirements. Optimum temperature. Range for growth.

Biochemical Reactions. Action on various carbohydrates, alcohols, proteins, inorganic salts, etc., are very important for identification purposes. Tests for the presence of certain end-products (*e.g.*, indole, acetylmethylcarbinol) are also made.

Pathogenicity and Serological Tests. Although not normally used for the saprophytic types, injection of laboratory animals and serological tests are important in the study of pathogenic bacteria.

For details concerning the methods for carrying out these tests reference should be made to the "Manual of Methods for Pure Culture Study," published by the Society of American Bacteriologists, or the numerous text books on practical bacteriology.

Classification. A number of different systems have been advanced for the classification of bacteria and each has had supporters with the result that some confusion exists, especially with regard to the nomenclature of bacteria. For example, the names *Escherichia coli* and *Bacterium coli* both refer to the same organism, while another species is variously known as *Salmonella typhimurium*, *Bacterium enteritidis* and *Bacillus paratyphosus B.* An historical survey of the various systems is given by Bergey and others (1939) in their "Manual of Determinative Bacteriology." The scheme for bacterial classification put forward in this Manual, although criticised on several grounds, has found general favour. Briefly summarised, the system is as follows :—

The class *Schizomycetes* is divided into seven orders, of which the first—*Eubacteriales*—includes all the bacteria concerned in food preservation. The order *Eubacteriales* consists of twelve families, each of which is further subdivided into tribes genera and species. Only the principal features of the families can be given here and these are :—

Cells not forming Spores.

1. *Nitrobacteriaceæ.* Autotrophic rod-shaped and coccal organisms. Some species are motile. Normally present in soil and water.

    Tribe I. *Nitrobacterieæ.*

        Genera. *Nitrobacter*, *Nitrosomonas*, *Nitrosococcus.*

    Tribe II. *Protobacterieæ.*

        Genera. *Hydrogenomonas*, *Methanomonas*, *Carboxydomonas.*

    Tribe III. *Thiobacilleæ.*

        Genus. *Thiobacillus.*

2. *Rhizobiaceæ.* Heterotrophic but able to use inorganic nitrogen. Rod-shaped. Generally found in soil and water but some species are parasitic.
   Genera : *Rhizobium, Chromobacterium, Alcaligenes.*
3. *Pseudomonadaceæ.* Straight or spiral rods, usually motile and found in soil and water. Some species are parasitic.
   Tribe I. *Spirilleæ.*
   Genera. *Vibrio, Cellvibrio, Cellfalcicula, Spirillum.*
   Tribe II. *Pseudomonadeæ.*
   Genera. *Pseudomonas, Phytomonas, Protaminobacter, Mycoplana.*
4. *Acetobacteriaceæ.* Rod-shaped cells but may show elongation, branching or swelling. They are characterised by oxidising alcohol to acetic acid.
   Genus. *Acetobacter.*
5. *Azotobacteriaceæ.* Large rods or cocci which utilise atmospheric nitrogen. Present in soil and water.
   Genus. *Azotobacter.*
6. *Micrococcaceæ.* Spheroidal cells, generally Gram-positive and non-motile. Cells show typical arrangement into pairs, tetrads, packets or irregular groups. They are frequently found in air and water. Some species are parasitic.
   Genera. *Micrococcus, Staphylococcus, Gaffkya, Sarcina.*
7. *Neisseriaceæ.* Gram-negative cocci. Strict parasites.
   Genera. *Neisseria, Veillonella.*
8. *Parvobacteriaceæ.* Small Gram-negative rods—usually parasitic.
   Tribe I. *Pasteurelleæ.*
   Genera. *Pasteurella, Malleomyces.*
   Tribe II. *Brucelleæ.*
   Genus. *Brucella.*

Tribe III. *Hemophileæ.*

Genera. *Hemophilus, Noguchia, Dialister.*

9. *Lactobacteriaceæ.* Gram-positive rods and cocci occurring singly and in pairs and chains. Produce lactic acid from carbohydrates. A few species also produce gas. Parasitic or saprophytic.

Tribe I. *Streptococceæ.*

Genera. *Diplococcus, Streptococcus, Leuconostoc.*

Tribe II. *Lactobacilleæ.*

Genera. *Lactobacillus, Propionibacterium.*

10. *Enterobacteriaceæ.* Gram-negative rods. Many species are parasitic. Attack carbohydrates with the production of acids or acids and gases.

Tribe I. *Escherichеæ.*

Genera. *Escherichia, Aerobacter, Klebsiella.*

Tribe II. *Erwineæ.*

Genus. *Erwinia.*

Tribe III. *Serrateæ.*

Genus. *Serratia.*

Tribe IV. *Proteæ.*

Genus. *Proteus.*

Tribe V. *Salmonelleæ.*

Genera. *Salmonella, Eberthella, Shigella.*

11. *Bacteriaceæ.* In this family are placed the non-sporing rods whose relationships to each other and to other groups have not been determined.

Genera. *Listerella, Microbacterium, Kurthia Cellulomonas, Achromobacter, Flavobacterium, Actinobacillus, Bacteroides, Fusobacterium, Bacterium.*

Cells forming Spores.

12. *Bacillaceæ.* Rod-shaped cells, usually Gram-positive. Frequently active in attacking proteins. Some species are parasitic.

Genera. *Bacillus* (aerobic), *Clostridium* (anaerobic).

True Fungi

The moulds and yeasts are frequently regarded as two distinct and separate groups of fungi. The distinction usually made is a morphological one, the moulds being defined as fungi which characteristically produce branched filaments (mycelium) and the yeasts as unicellular fungi. No true distinction exists however, since some moulds assume a yeast-like form under certain conditions while a number of normally unicellular yeasts may give rise to mycelium. For the present purpose however, it is convenient to consider the moulds and yeasts separately.

Moulds

The common moulds consist of tubular filaments termed hyphæ which, being profusely branched, intertwine to form a web-like structure known as mycelium. In many cases the mycelium can be differentiated into a vegetative portion which extends over and into the medium and a fertile, spore-bearing portion extending into the air. Hyphæ may be septate or non-septate. In the former case each hypha is divided by transverse walls or septa into a number of cells each containing one or more nuclei. Non-septate hyphæ have the appearance of continuous, multi-nucleated tubes. Extension of the hyphæ and growth of mycelium takes place either by growth at the tips (apical growth) or by division of the cells in the hyphæ (intercalary growth).

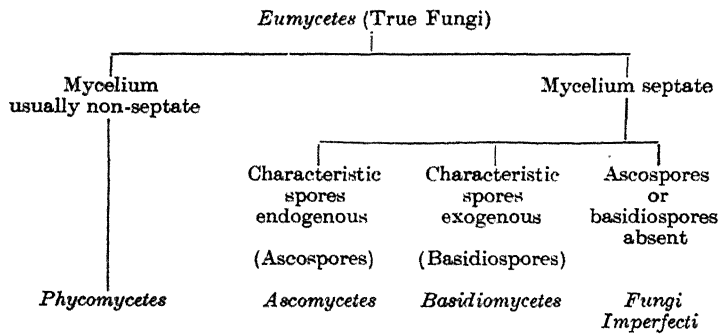
Reproduction. Moulds normally reproduce by means of spores which are formed in large numbers by a single plant. Sporulation in moulds is a truly reproductive process, each spore being capable of giving rise to a new growth by emitting one or more hyphæ. Under favourable conditions the cycle from spore to spore may be completed fairly rapidly (24 to 48 hours). Mould spores resemble bacteria spores in that they are resistant to adverse conditions.

The identification of moulds is largely based on the

characteristics of the reproductive spore systems which may be sexual or asexual in origin. In many species both types of spore formation occur but if sexual spores are present they are regarded as the " perfect " form and are primarily important for classification purposes; asexual spores are termed " imperfect " or " accessory " but they are important in the differentiation of the species even in those types which form sexual spores. Sexual sporulation is always preceded by the fusion of two specialised cells and their nuclei, but in asexual spore-formation there is no preliminary fusion of cells, the spores being produced at the ends of fertile hyphæ. In some species asexual spores arise singly or in groups as a result of budding or segmentation of the fertile hyphæ. Such spores are termed conidia and the hyphæ which bear them are conidiophores. Conidia may be attached to fertile hyphæ by short stalks or sterigmata which, in some species, are branched. Asexual spores may also be produced within a sac or sporangium. Such spores are sporangiospores and the hyphæ bearing them are termed sporangiophores. The tip of the sporangiophore which projects into the sporangium is known as the columella and its shape and size are important for identification purposes. Sporangia are never produced by the septate moulds. In some species of fungi the hyphæ break up into short segments, each of which is regarded as a spore since it is capable of giving rise to a new growth. The name Oidia is given to these segments.

Asexual spores known as chlamydospores are formed by a thickening and swelling of the hyphal cells. They occur in any portion of the mycelium and are not confined to special areas. Such spores are very resistant to unfavourable conditions. In some species hyphæ combine to form hard compact masses or balls termed sclerotia, which are also able to survive adverse conditions.

Principal Groups. True fungi are usually separated on the basis of the following characteristics:—



Phycomycetes. This group is separated into two classes, the *Oomycetes* and the *Zygomycetes.* From the canning point of view members of the first class are unimportant.

In the *Zygomycetes,* the sexual spores are zygospores. They are formed as the result of two hyphæ coming into contact. At the point of contact protuberances or progametangia develop, each of which becomes separated from its parent hypha by the formation of a septum. The cut off portions which remain in contact fuse and the wall of the resulting cell becomes thick and warty. This group includes species belonging to the genera *Mucor, Rhizopus,* and *Thamnidium,* which are frequently associated with food spoilage.

Ascomycetes. This group is characterised by the formation of sexual ascospores within a sac or ascus. There are usually eight ascospores within each ascus, many of which may be formed in a single growth. There is great diversity in the manner in which asci are formed by the various species. In the simplest forms as in yeasts, there first occurs a fusion of two adjacent cells and their nuclei which divide into daughter nuclei, each of which becomes enclosed by a spore wall. In this instance, the yeast cell forms the ascus. In higher members of the group, a fusion of two cells occurs and from the resulting cell new filaments or ascogenous hyphæ arise in which asci containing ascospores are developed. In some species groups of asci become surrounded by

a dense, protective, mass of filaments to form a fruiting body known as an ascocarp. If the ascocarp is globular and closed it is known as a perithecium, if cup-shaped and wide open it is an apothecium. Spores produced in asci are termed endogenous. Some species of the *Aspergillus* and *Penicillium* genera, important in food spoilage, form asci and should, therefore, be classified with the *Ascomycetes* but in the great majority sexual spores are unknown and these species are accordingly grouped with the *Fungi Imperfecti*. One species, belonging to the Ascomycetes, *Byssochlamys fulva*, is an important spoilage agent in canned fruits.

Basidiomycetes. This group includes the mushrooms, toadstools and parasitic rusts and are not important in food microbiology. The characteristic spores from which the group derives its name are the basidiospores.

Fungi Imperfecti. This class includes all the species in which sexual or "perfect" sporulation is unknown and which reproduce by means of asexual spores. The great majority of the common moulds belong to the *Fungi Imperfecti*. Important genera associated with food spoilage include *Aspergillus*, *Penicillium*, *Sporotrichum*, *Cladosporium*, *Alternaria*, *Fusarium*, *Oidium* and *Monilia*.

Many species of mould are of major industrial importance. The production of citric acid by *Aspergillus niger* and the ripening of cheeses by species of *Penicillium* are examples. Detailed information concerning the moulds is given by Henrici (1938) and Gwyne-Vaughan and Barnes (1937). Smith's "Introduction to Industrial Mycology" contains full descriptions of the species commonly occurring in industry.

Yeasts

Henrici (1941) states as a fairly satisfactory definition that yeasts are fungi with nuclei in which the usual growth form is unicellular. A yeast cell consists of a permeable cell-wall or membrane enclosing a cytoplasm containing a

nucleus and various granules. In young cells, the cell wall is thin and the cytoplasm relatively clear but as the cell ages, thickening of the wall occurs and granules and vacuoles appear in the cytoplasm. The nucleus is not ordinarily visible except in the stained cells. Yeast cells are mostly detached from each other except when actively growing and are of various shapes, the usual form being round, oval or elliptical. In size they measure up to 8μ in diameter and 10μ in length.

Reproduction. Reproduction is accomplished by fission, budding or spore formation. Spore formation in yeasts does not occur with the same facility as that in bacteria or moulds and special methods are frequently necessary to induce sporulation. In multiplication by fission the cell divides into two daughter cells, the process being similar to that which takes place in the bacteria. In budding, a bulge or bud appears in the cell wall. This bud, which becomes constricted at its base, enlarges to approximately the same size and shape of the parent cell and finally becomes separated from it. Frequently the bud cells give rise to new buds before they become detached from the parent cell so that a group of connected cells of varying size is formed. As with the moulds, spore formation in yeasts may be sexual or asexual. In the sexual process there may be conjugation of two separate cells, the nuclei of which fuse and divide, in some cases repeatedly. Condensation of cytoplasmic material takes place round the daughter nuclei and each becomes enclosed by a spore wall. This type of conjugation is termed isogamic. In heterogamic conjugation the nucleus of a bud cell fuses with the nucleus of the parent cell. Asexual spores result from the nucleus of the cell undergoing division but the process is not preceded by any preliminary fusion of cells or nuclei. In the great majority of yeasts spores are endogenous, but in certain types spore formation is exogenous. The spores of yeasts are generally multiple and are more resistant to unfavourable conditions than the

vegetative cell but, as in the case of mould spores, the difference is not so marked as between bacteria and their spores.

Principal Groups. Differentiation of yeasts is principally based on their methods of reproduction but biochemical reactions, cultural characteristics and other features are of diagnostic importance. The technical procedures used in the identification of yeasts are outlined by Henrici (1941). The yeasts are often grouped into two families, those forming spores being placed in the *Saccharomycetaceæ* and the non-sporing varieties in the *Torulaceæ*. These groups are also respectively known as true yeasts and false yeasts. Strictly, the grouping is unsatisfactory but is widely used in industry. A number of improved systematic classifications have been made and for details of these the reader is referred to the review by Henrici. The industrially important genera of yeasts include the following :—

Saccharomycetaceæ (Spore-forming yeasts). *Saccharomyces*. This genus includes many important species of which *Saccharomyces cerevisiæ*, the brewers' and bakers' yeast is the most notable. It has marked ability to produce alcohol and carbon dioxide in quantity from sugar. Characteristic cells are round and reproduction takes place by budding and asexual spores. Other important species are *Saccharomyces ellipsoideus* (used in wine fermentation) and *Saccharomyces pastorianus* which occurs as a contaminant producing off flavours in the brewing industry.

Zygosaccharomyces : Reproduced by means of budding and sexual spores (isogamic). These yeasts are important in food preservation because of their activity in fermenting concentrated sugar products.

Pichia and *Willia :* These genera are important as contaminants in the brewing industry. They form esters (ethyl acetate, amyl acetate, etc.) from sugars but not alcohol. When grown in liquids they form dry, wrinkled pellicles. Such films are found on the surface of pickle brines.

Torulaceæ (Non-spore forming Yeasts). Harrison (1928) divides these yeasts into four groups : *Mycotorula* (producing rudimentary mycelium), *Torula* (no rudimentary mycelium), *Rhodotorula* (red pigment formed) and *Chromotorula* (pigment other than red formed). These yeasts are widely distributed in soil and on fruit, cereals, etc., and are mainly important as spoilage agents in the brewing, dairy and other food industries. Some form films or pellicles on liquids and are very common on pickle brines.

Biological Characteristics of Fungi. The fungi are devoid of chlorophyll and cannot synthesise their food solely from inorganic materials. All are, therefore, heterotrophic and require an organic source of carbon for energy purposes and a few appear to require organic nitrogen. In addition to oxygen, other elements known to be essential in the nutrition of fungi are potassium, phosphorus, sulphur, magnesium and in some cases, iron. Usually inorganic sources of these are utilisable. Moulds are distinguished for their ability to thrive on minute quantities of food materials.

Fungi obtain their nutrients by absorption through the cell wall, as do the bacteria. They are possessed of a wide range of enzymic activity, carbohydrates of varying complexity, proteins, fats and organic acids being readily attacked by various species. The principal products of fermentation by fungi are non-volatile acids and alcohol. Carbon dioxide may be produced but not hydrogen or methane and in this respect the fungi differ from bacteria, which frequently produce hydrogen, and less frequently, methane.

In their oxygen requirements the majority of the common moulds are strict aerobes but some species are capable of growth under markedly reduced oxygen tension. Many yeasts are facultative anaerobes but according to Smith (1938) no truly anaerobic fungus exists.

The temperature relationships of the fungi vary widely. The optimum temperature for growth of most fungi lies

between 25 and 30 deg. C (77–86 deg. F.). Many species fail to develop above 30 deg. C. (86 deg. F.) but a few have their optimum above this temperature. The mould *Aspergillus fumigatus* can exist at temperatures above 50 deg. C. (122 deg. F) although spore formation and optimum growth occur at 40 deg. C. (104 deg. F.). The optimum temperature for mycelial development does not necessarily coincide with that for other purposes such as sexual spore production. The lower limit for growth varies but development of some species such as *Cladosporium herbarum* takes place at about — 6 deg. C. (21 dec. F.).

The fungi also show considerable variation in their resistance to heat. Spores are generally slightly more resistant to heat than vegetative cells but are not nearly so resistant as bacterial spores. Some species are destroyed by long exposure to temperature over 30 deg. C. (86 deg. F.) and most species are destroyed by 30 minutes heating at 65 deg. C. (149 deg. F.). An exception is the mould *Byssochlamys fulva* which causes spoilage in canned fruits. The spores of this species survived heating for 30 minutes at 87–88 deg. C. (188·6–190·4 deg. F.). Recently, Williams, Cameron and Williams (1941) have reported that the sclerotia of a *Penicillium* species required 300 minutes exposure at 85 deg. C. (185 deg. F.) for destruction.

REFERENCES

ANDERSON, C. G. 1938. "Introduction to Bacteriological Chemistry." E. and S. Livingstone, Edinburgh.

BERGEY, D. H. 1939. "Manual of Determinative Bacteriology." Baillière Tindall and Cox, London.

CLEARY, J. P., BEARD, P. J., and CLIFTON, C. E. 1935. *J. Bact.*, **29**, 205.

DARANYI, J. 1930. *Biol. Zentralbl.*, **50**, 471.

DORN, F. L., and RAHN, O. 1939. *Arch. Mikrobiol.*, **10**, 6.

GWYNNE-VAUGHAN, H. C. I., and BARNES, B. 1937. "The Fungi." Univ. Press, Cambridge.

HAINES, R. B. 1934. *J. Hyg.*, **34**, 277.

HARRISON, F. C. 1928. *Trans. Roy. Soc. Canada*, **22**, 187.

HENRICI, A. T. 1938. "Moulds, Yeasts and Actinomycetes." Chapman and Hall Ltd., London.

HENRICI, A. T. 1941. *Bact. Reviews*, **5**, 97.

HESS, E. 1934. *Contr. Canadian Biol. Fish.*, **8**, 491.
HEWITT, L. F. 1936. "Oxidation-Reduction Potentials in Bacteriology and Biochemistry." London County Council.
KAPLAN, I., and WILLIAMS, J. W. 1941. *J. Bact.*, **42**, 265.
KNAYSI, G. 1941. *J. Bact.*, **41**, 141.
KNIGHT, B. C. J. G. 1936. "Bacterial Nutrition." *Med. Res. Council, Spec. Rept.*, No. 210, H.M.S.O., London.
KOSER, A. S., and SAUNDERS, F. 1938. *Bact. Reviews*, **2**, 99.
LEWIS, I. M. 1941. *Bact. Reviews*, **5**, 181.
POLEVITSKY, K. 1941. *J. Bact.*, **41**, 260 (Abst.).
SMITH, G. 1938. "Introduction to Industrial Mycology." Arnold & Co. Ltd., London.
SOCIETY OF AMERICAN BACTERIOLOGISTS. "Manual of Methods for Pure Culture Study." Geneva, N.Y.
TOPLEY, W. W. C., and WILSON, G. S. 1936. "Principles of Bacteriology and Immunity." Arnold & Co. Ltd., London.
WALKER, H. H. 1932. *Science*, **76**, 602.
WILLIAMS, C. C., CAMERON, E. J., and WILLIAMS, O. B. 1941. *Food Res.*, **6**, 69.
WINSLOW, C. E. A., and WALKER, H. H. 1939. *Bact. Reviews*, **3**, 147.

CHAPTER II

CONTROL OF SPOILAGE MICRO-ORGANISMS

Two general procedures are adopted in safeguarding food against spoilage by micro-organisms. The first, on which the canning operation is based, is sterilisation. This involves exposing the food to heat in order to destroy spoilage organisms, the food being protected against subsequent contamination by being enclosed in an airtight container. The second procedure aims at preservation by inhibiting the development of spoilage organisms and this may be achieved by various methods, the food being so treated that the activities of the organisms are inhibited or retarded. Preservation by such methods does not necessarily imply the destruction of the organisms (*i.e.*, a germicidal or fungicidal effect) and on removal or decrease of the inhibiting influence the food will undergo spoilage. It is necessary to give some consideration to the more frequently used methods of preservation other than heat since food preserved by such methods is largely used as raw material for canning; furthermore, some canned and bottled products such as fruit, jam, sauces and pickles are preserved by the use of both heat and inhibitory substances. The chief methods which are applied commercially include refrigeration, dehydration, filtration, pickling or curing, fermentation, smoking, and the addition of the so-called natural preservatives—sugar, salt, acid and spices—or of chemical preservatives such as sulphur dioxide and benzoic acid. Some of these methods are applied jointly, their effects being additive.

Refrigeration

Low temperatures preserve foods by retarding or preventing the growth of spoilage organisms and, in the case

of fresh foods, by inhibiting the action of natural autolytic enzymes.

The organisms growing at or below 0 deg. C. (32 deg. F.) are those with an optimum temperature in the region of 15–20 deg. C. (59–68 deg. F.), species whose optimum lies at about 37 deg. C. (98·6 deg. F.) developing very slowly, if at all, below 5 deg. C. (41 deg. F.). The "cold store" or psychrophilic types are capable of comparatively rapid growth at 0 deg. C. (32 deg. F.) and although their rate of growth is slower than that at higher temperatures the total number of cells produced may be greater (Hess, 1934). The micro-organisms commonly developing at low temperatures include bacteria belonging to the genera *Achromobacter*, *Flavobacterium*, *Pseudomonas*, and *Micrococcus*, yeasts of the Torula type and mould species of the genera *Penicillium*, *Cladosporium*, *Mucor* and *Thamnidium*.

According to Haines (1934) the limiting temperature for growth of the psychrophilic group of organisms including bacteria, yeasts and moulds, lies between — 5 and — 10 deg. C. (23–14 deg. F.) and is probably near — 7 deg. C. (19 deg. F.). Lea (1931) found that storage at — 5 deg. C. (23 deg. F.) failed to prevent the development of yeasts and moulds on frozen meat, colonies appearing after 7 weeks. Berry and Magoon (1934) reported the growth of *Psuedomonas*, *Lactobacillus*, *Torula*, *Monilia* and *Penicillium* species at — 4 deg. C. (25 deg. F.) and *Cladosporium* and *Sporotrichum* species at — 6·7 deg. C. (20 deg. F.). The lower limit at which the growth of micro-organisms occurs is not determined by temperature alone, a very important factor being the amount of water frozen out of the substrate (Haines, 1934). Where there is actual ice formation the growth of bacteria is retarded whilst moulds and yeasts preponderate because they are better able to withstand the higher osmotic pressure which results from the concentration of solutes following the separation of the water as ice. For this reason the growth of bacteria on super-cooled media

occurs at a lower temperature than growth on frozen media. Bacterial growth on frozen media ceases at about — 3 to — 4 deg. C. (27–25 deg. F.).

Exposure to low temperature causes an initial decrease in the numbers of living bacteria followed by a lag period before development of the survivors occurs, the extent of the initial decrease and the lag depending on the temperature. With bacteria in fish muscle, Stewart (1934) found the initial reduction in numbers was slight between — 1·2 deg. and — 3 deg. C. (30–27 deg. F.) and after a lag period of 1 to 15 days, rapid multiplication occurred. At — 4 deg. C. (25 deg. F.) the initial decrease was greater and the lag phase extended to 35 to 50 days, while at — 6 deg. C. (21 deg. F.) initial decrease was marked and the lag even more prolonged. At — 12 deg. C. (10 deg. F.), however, the inital decrease was less than at — 6 deg. C. (21 deg. F.), but there was no sign of subsequent increase after 210 days. Haines (1934a) noted that death of bacteria occurred more rapidly at — 1 to — 5 deg. C. (30–23 deg. F.) than at — 20 deg. C. (— 4 deg. F.) and McFarlane (1941) observed a similar effect, bacteria and yeasts both undergoing greater destruction at — 10 deg. C. (14 deg. F.) than at — 20 deg. C. (— 4 deg. F.).

In general, micro-organisms are remarkably resistant to low temperatures, even pathogenic types which cannot under any circumstances be considered as psychrophilic surviving for long periods. McClesky and Christopher (1941) investigating pathogenic bacteria in " quick frozen " strawberries (— 18 deg. C. (0 deg. F.) found that *Eberthella typhosa* survived for six months, *Staphylococcus aureus* for five months and *Salmonella* types for one month. Smart (1934) found that many species of bacteria and several species of moulds and yeasts survived for three years in frozen strawberries.

Refrigeration may be combined with gas storage. It has been well established that carbon dioxide gas has an inhibitory action on the organisms developing at cold-store temperatures and this circumstance has led to the use of

the gas in conjunction with cold storage for meat and eggs. Chilled beef stored in air containing 10 per cent. carbon dioxide remains free from microbial spoilage for 60 to 70 days (Moran, 1938). Complete inhibition of growth may be obtained with higher concentrations of gas but this is offset by the deterioration which takes place in the quality of the meat (loss of bloom).

Moisture Limitation

Under this heading dehydration and the addition of sugar may be considered jointly since both methods have for their object the reduction of available moisture to a level where development of micro-organisms is prevented.

With the exception of the osmophilic yeasts which are a special problem, moulds are less exacting in their moisture requirements than other organisms. It follows, therefore, that for successful preservation, foods must contain less moisture than the minimum which will permit mould development. The total amount of water in a product is not necessarily a true indication of its liability to mould attack, the controlling factor being the availability of the water to the mould. In a product such as jam, moisture is not normally available for mould growth, whereas materials such as cereal products with much lower moisture contents may undergo spoilage because the water present is available to the mould. The availability of the water is most conveniently expressed in terms of the relative humidity of the atmosphere in equilibrium with the product.

According to Galloway (1935) the minimum relative humidity necessary for development of the commoner moulds varies with the species from 75 to 95 per cent., *Aspergillus* and *Penicillium* types being the most resistant to low humidity conditions. Barton-Wright and Tomkins (1940) have shown that the critical humidity for mould growth in flour increases as the temperature decreases; at 20 deg. C. (68 deg. F.), mould was inhibited at 77 per cent.

R.H. (moisture 16 per cent.). At 15 deg. C. (59 deg. F.) 82·5 per cent. R.H. (moisture 16·5 per cent.) was inhibitory and at 5 deg. C. (41 deg. F.), 85 per cent. R.H. (moisture 17·4 per cent.) sufficed. Other factors such as the presence of toxic substances and the nutritional value of the product for moulds can be expected to influence the limiting humidity but it may be said that foods in which the relative humidity lies below 75 per cent. are generally resistant to mould spoilage. Peas, cereals and similar dry goods should, therefore, be dehydrated until the humidity of the atmosphere in equilibrium with the products is below this limit. Similarly in sugar preserves, the solutes must be present in sufficient concentration to reduce the humidity to the inhibitory level. For equal concentrations, the osmotic pressure of sugars in solution is highest with those sugars of low molecular weight. Since the relative humidity of a solution decreases with increase in osmotic pressure, monosaccharides such as dextrose and fructose are more effective in reducing humidity than sucrose. Thus a preserve containing 65 per cent. sugar as sucrose will be more liable to mould attack than a similar product with 65 per cent. total sugars of which a proportion is present as invert sugar. Erickson and Fabian (1942) have recently investigated the preserving action of various sugars. They found that for bacteria the order of effectiveness was fructose > dextrose > sucrose > lactose. Thermophiles were more susceptible to the action of sugars than streptococcal species. For yeasts, fructose and dextrose were equally effective in 5 to 15 per cent. lower concentration than sucrose.

The osmophilic yeasts are able to tolerate very high concentrations of sugar and cause spoilage in honey, chocolate centres, jam, molasses and similar products in which the sugar content may be as high as 80 per cent. Members of the genus *Zygosaccharomyces* appear to be the most active spoilage agents. According to Pouncy and Summers (1939), confectionery products with a relative

humidity of less than 69 per cent. are resistant to spoilage by osmophilic yeasts. These authors devised an ingenious method of estimating the relative humidity of confectionery by means of the deliquescing of various crystals when exposed to air in equilibrium with the material. With material poor in protein the critical humidity for fermentation was considerably higher than that in protein-rich material. They point out that when products have a humidity above the critical point this can in many cases be effectively lowered by the addition of 10 per cent. invert sugar. Spoilage in honey in which the sugar content is about 80 per cent., mostly as invert sugar, has been investigated by a number of workers. Fabian and Quinet (1928) suggest that the fermentation of such a highly concentrated product as honey is due to its hygroscopic action, sufficient water being absorbed at the surface to allow fermentation to begin. Yeasts subsequently become gradually accustomed to the high sugar concentration and eventually grow throughout the honey. The subject of honey fermentation has been fully reviewed by Wilson (1934).

With the majority of canned foods, preservation by control of the moisture content is not possible. There are, however, some canned and glass packed products for which moisture control is applied. These include cereals such as oatmeal, semolina and such sugar products as jam, candied fruit peel, confectionery and sweetened condensed milk. This latter product is invariably unsterile but the organisms normally present are unable to develop. With some types of jams and marmalade (as in some American products) where the sugar content is low (*i.e.*, about 60 per cent.) it is necessary to give heat treatment to prevent spoilage.

Salt

The action of salt in preserving food is not fully understood but it does not appear to act solely by virtue of its osmotic effect.

The concentration of salt necessary to inhibit the growth of micro-organisms in food is related to many factors. These include the water content, type of infection, *p*H, temperature, protein content, and presence of inhibitory substances such as acids. The water content is obviously of major importance since it is the concentration of salt in the water phase and not the amount in the food as a whole which is significant. According to Labrie and Gibbons (1937), the preserving action of salt on bacteria increases with decreasing temperature from 21 to 10 deg. C. (69·8–50 deg. F.). Callow (1929) also noted that the amount of salt required to inhibit mould growth decreased with decreasing temperature, 8 per cent. sufficing at 0 deg. C. (32 deg. F.) whereas 12 per cent. was necessary at room temperature. The influence of the composition of the medium on the salt tolerance of micro-organisms has been repeatedly demonstrated. Garrard and Lochhead (1939) observed that organisms exhibited greater salt-tolerance in curing pickle than in broths of similar salt content. Stuart (1940) showed that growth of halophilic bacteria may be either stimulated or retarded by varying the protein content of the medium. The effect of *p*H on salt tolerance was studied by Joslyn and Cruess (1929) who found that lowering the *p*H caused a marked decrease in the salt tolerance of various yeast and mould species.

Schoop (1929) suggested that bacteria may be separated into three groups, based on their relationship to salt, as follows :—

(1) Non-halophilic—not showing growth in high salt concentration.

(2) Obligate halophiles—growing only in high salt concentration.

(3) Facultative halophiles, which develop in high or low salt concentration.

The work of Stuart and James (1938), however, tends to discount the existence of a truly obligate halophilic group. None of the so-called obligate halophiles examined by them

failed to develop on low-salt media if cultures allowed to age for 30 days or more were utilised as inoculum. Stuart (1938) has also shown that, contrary to the general view that halophilic bacteria are exclusively associated with salt environment such as solar salt, sea-water, fish, etc., they are in fact widely distributed in nature and can be isolated in 25 per cent. salt media from non-salt environments, including stagnant water, sulphur springs, rat-dung and soil, provided an incubation period up to 90 days is permitted.

From the wide diversity of the types reported in the literature, it is evident that a typical halophilic flora does not exist, many organisms of a widely varying morphological and biochemical character being represented. Micro-organisms show such variation in their ability to tolerate salt that growth of one species or another occurs throughout the range up to saturation. Pathogenic organisms generally are more susceptible to the action of strong salt solution than are the saprophytic types. Karaffa-Korbutt (1912) found that the growth of the colon group was inhibited by 8 to 9 per cent. salt. Nunheimer and Fabian (1940) state that sodium chloride in 15 to 20 per cent. concentration prevents the growth of some strains of food-poisoning staphylococci while 20 to 25 per cent. has a definite lethal effect. Livingstone (quoted by Buchanan and Fulmer, 1930) pointed out that the spherical form presents the least surface for water exchange and is, therefore, advantageous in concentrated solutions and it is interesting to note that as a group, micrococci generally exhibit a high salt-tolerance, many types developing freely in the presence of 25 per cent. salt.

Many of the bacterial species growing in strong salt solutions are chromogenic and cause spoilage by producing discolouration on salted fish and hides. Such spoilage has been reported by Harrison and Kennedy (1922), Cloake (1923), Robertson (1931) and others. A non-sporing anaerobic rod isolated and described by Baumgartner (1937)

developed in media saturated with salt. This organism is the cause of gaseous spoilage in unsterilised salted fish products such as pastes and fish sauces ; it can be effectively controlled by reducing the *p*H of such products to below 5·5.

Karaffa-Korbutt and others have observed that the growth of yeasts is not prevented by 25 per cent. salt. Film-forming yeasts examined by Mrak and Bonar (1939) grew in brines with 24 per cent. salt. Yeasts of this type grow on the surface of pickled vegetable brines and by oxidising the lactic acid produced during fermentation of the vegetables, impair their keeping quality. Moulds may also show this undesirable activity. According to Tanner (1932) mould growth may occur in the presence of 20–30 per cent. salt. Species of *Penicillium* studied by Joslyn and Cruess (1929) developed in 20 per cent. salt brines.

Acids

The effect of acids in preventing the development of micro-organisms may be due to the hydrogen ion concentration or to the toxicity of the undissociated molecule or the anion. With mineral acids the toxic effect is related to the hydrogen ion concentration but the toxicity of the organic acids is disproportionate to the degree of dissociation and is due mainly to the undissociated molecule or the anion.

The yeasts and moulds are very much less susceptible to the effect of high hydrogen ion concentration than bacteria. Most species of bacteria have their optimum *p*H in the region of neutrality and are unable to develop below about *p*H 4·5. Among the most acid-tolerant bacteria are those of the *Lactobacillus* and *Clostridium butyricum* groups growing down to about *p*H 3·5, whereas moulds and yeasts which develop best in the slightly acid range (*p*H 5·0–6·0) may tolerate *p*H 2·0 or even lower.

In the preservation of foods, acetic and lactic acids are those most commonly employed. Pederson and Breed (1926) found that 1 per cent. acetic acid prevented spoilage

in tomato catsup and that combinations of salt and sugar with the acid did not lower appreciably the amount of acid required. Fabian and Wadsworth (1939) found acetic acid to be a better preservative than lactic acid for pickles and Levine and Fellers (1940) have also reported that acetic is more toxic than lactic acid for bacteria, yeast and mould species. When the reaction of the medium was adjusted with acetic acid, bacteria were inhibited at *p*H 4·9, *Saccharomyces cerevisiæ* at *p*H 3·9 and *Aspergillus niger* at *p*H 4·1, the titratable acidities being 0·04 per cent., 0·59 per cent. and 0·27 per cent. respectively. It should be noted that the acidities mentioned relate to inhibition of a few species in laboratory media and that in commercial practice higher concentrations of acetic acid (*e.g.*, 1·5–2 per cent.) are required to prevent spoilage in products such as sauces, pickles, etc. In a later report, Levine and Fellers (1940a) noted that the addition of 5 per cent. salt or 20 per cent. sugar did not appreciably reduce the amount of acid required to prevent growth. In non-toxic concentration acetic acid stimulated the growth of the mould by serving as a source of energy. Erickson and Fabian (1942) found that when based on *p*H value, the order of preserving and germicidal power for bacteria was acetic > citric > lactic acid. When judged by the amount of acid, the order was lactic > acetic >citric. For yeasts, the order was acetic > lactic > citric, irrespective of whether it was based on *p*H value or concentration of acid. Yeasts were more tolerant of acids than were bacteria. They also observed that a combination of sugar with the preserving quantity of acid rendered the mixture germicidal. A similar effect was observed by Nunheimer and Fabian (*loc. cit.*). They found that the amount of dextrose required to exert a germicidal action on staphylococcal strains could be reduced to 50 per cent. when used in the presence of half the inhibitory concentration of acid. Salt and sucrose could be reduced only by 30 and 20 per cent. respectively and still bring about a

germicidal effect. The fungistatic action of the fatty acids was studied by Hoffman, Schweitzer and Dalby (1939). Over the *p*H range of 2–8 many of these acids were effective in preventing mould growth. Below *p*H 5·0 acetic acid was very effective, the amount required decreasing with *p*H. At *p*H 2·0, less than 0·04 M acetic acid was sufficient, whilst at *p*H 5·0, between 0·08 M and 0·12 M concentration was necessary. At the same *p*H, propionic acid was effective in lower concentrations than acetic acid and was active up to *p*H 6·0–7·0. Propionic acid and its salts have been widely recommended for the prevention of spoilage in foodstuffs, but their use might be judged contrary to the British preservative regulations. In bread, calcium propionate has been found effective in preventing the condition known as "ropiness." Olsen and Mady (1940) reported that propionic acid inhibited surface mould growth on butter. The acid was more effective than the calcium salt and the latter was more effective than the sodium salt. The *p*H of the substrate was an important factor. Calcium propionate in 0·1–0·5 per cent. concentration has been found to be efficacious in preventing mould growth on fruit jellies, glaze jelly and similar products.

Chemical Preservatives

Public Health regulations define a "preservative" as any substance capable of inhibiting, retarding or arresting the process of fermentation, acidification or other decomposition of food or of masking any evidence of putrefaction. The Regulations exclude such substances as salt, saltpetre, sugar, lactic or acetic acids, glycerine, alcohol, spices, essential oils and herbs. A great many chemical agents have a preservative action by virtue of the fact that they combine with microbial protoplasm, thereby producing a toxic effect on the cell. The action is not limited to microbial protoplasm but occurs with protoplasm in general and substances that are toxic to micro-organisms are generally harmful to the

tissues of the body. It is for this reason that, with very few exceptions, the addition of preservatives to food is prohibited in Great Britain. In this country, the permissible preservatives are sulphur dioxide (including sulphite) benzoic acid (including benzoates) and sodium nitrite ; these may only be used in strictly controlled accounts for specified foods. Boric acid (including borates) may be added under licence to bacon and margarine but this is presumably only a war-time measure.

The action of preservatives is influenced to a marked degree by a number of factors, a full discussion of which is outside the scope of this book but a brief statement may indicate their practical importance. The activity of a preservative is primarily dependent on its concentration. If present in adequate concentration the effect on micro-organisms is lethal. In lower concentration inhibition of growth but not death results, while in very low concentration the toxic effect is lost completely and development of the organisms is stimulated. The degree of dilution required to produce these changes in activity varies with each preservative so that if two different preservatives are diluted in the same proportion the effect on their toxicity may be quite different. A numerical expression, the concentration coefficient, is used to denote the effect of dilution on the activity of a preservative.

Temperature has an important effect on the activity of preservatives. In general, toxicity is markedly increased by rise in temperature, the increase varying with different substances. The degree to which toxicity is increased by a given rise of temperature is expressed as the temperature coefficient. In addition to the effect of temperature on the activity of the preservative, the effect on the organisms has also to be considered. Where the concentration of preservative is only sufficient to cause inhibition of growth the stimulatory effect on the organisms of a slight rise in temperature may outweigh the effect on the activity of the

preservative. At temperatures in excess of the maximum for growth, however, very small amounts of preservatives may exert a pronounced lethal effect.

The type of organism and the number present are factors requiring consideration. As with other harmful agencies, the spores of micro-organisms are more resistant to toxic chemicals than vegetative cells. A given preservative cannot be relied upon to be equally effective against all types of organisms, even different strains of the same species varying considerably in their resistance to the same preservative. For example, Burgvits (1933) found that the concentrations of sulphur dioxide required to inhibit the development of three strains of *Saccharomyces ellipsoideus* in 1 litre of wine must were 186–290 mgm., 289–480 mgm., and more than 480 mgm. The number of cells present may influence the efficacy of a preservative and a concentration which is adequate to control a slight infection may be inadequate to deal with a large number of organisms. In this connection it is clearly necessary to keep contamination of preserved foods to a minimum.

In addition to these factors the nature of the food to which the preservative is added is very important. Hydrogen-ion concentration has a marked effect, the toxicity of most preservatives being greatly increased in acid medium. Cruess (1932) has reported that the concentrations of various preservatives required to prevent spoilage are much less for acid than for non-acid foods. In fruit juices at *p*H 7·0 as much as 300 times the concentration of certain preservatives was required to prevent growth of various organisms as at *p*H 3·0–3·5. At neutrality, about 4 per cent. sodium benzoate was necessary to inhibit growth, whereas at *p*H 2·3–2·4, 0·02–0·03 per cent. sufficed. A similar effect was shown with other preservatives, including sulphite.

In the presence of organic matter the action of most preservatives is retarded. Apart from other causes, a

preservative may react with organic matter to form compounds which are inert or less toxic than the free preservative. Cruess (1938) states that sulphur dioxide combines with sugar and other compounds in fruit juice and that the combined form has very little preserving power, 6,000 parts per million being less toxic than 50 parts per million of free sulphur dioxide.

For full information concerning the process of controlling micro-organisms by chemicals, McCulloch (1936) and Topley and Wilson (1936) should be consulted.

Spices

The preservative action of the spices and herbs used for flavouring foods has received considerable attention and some of them have been shown to possess definite preservative properties. In all cases the preservative effect is due to the essential oils. Most workers have come to the conclusion that cloves, cinnamon and mustard exert a greater preservative action than other spices and herbs. Corran and Edgar (1933), who cite earlier work on the subject, investigated the action of various spices, herbs and their oils on yeast (*Saccharomyces cerevisiæ*). They found that brown mustard flour was the most potent followed by cloves and cinnamon. Cardomoms, cummin, corianders, caraway, celery seed, pimento, nutmeg, ginger, marjoram and other spices and herbs had little or no effect. Similarly, volatile oil of mustard was found to have a greater preservative action than the essential oils of other spices and herbs. Volatile oil of mustard in a concentration of 200 parts per million or 5,000 parts per million of brown mustard flour were more potent than sulphur dioxide and benzoic acid in concentrations of 350 and 600 parts per million respectively. Fabian, Krehl and Little (1939), using a number of bacterial species as test organisms, concluded that there was considerable variation in the resistance of different organisms to the same spice and of the same

organism to different spices. Their results showed that ground cinnamon and cloves were the only spices which were inhibitory in low concentration for bacteria. Ground pepper-corn and allspice were inhibitory in 1 per cent. concentration and mustard, mace, nutmeg and ginger in 5 per cent. concentration. 0·1 per cent. of a 50 per cent. emulsion of oil of mustard gave some inhibition, while 1 per cent. of a 50 per cent. emulsion was completely inhibitory.

These and other investigations show that the preservative action of some spices can be of considerable practical importance. The effect of elevated temperature on the toxic action of spices on micro-organisms does not appear to have been investigated but it would seem possible that at the temperatures used for processing canned and glass-packed foods, their action may be such as to cause a reduction in the heat resistance of micro-organisms.

The Curing of Meat

In addition to imparting desirable colour and flavour, the curing of meat has an appreciable preservative effect. The theories underlying the formation of colour in cooked cured meats have been discussed by Urbain and Jensen (1940). According to them, muscle pigment—myo-hæmoglobin combines with nitric oxide to form nitric oxide myo-hæmoglobin which is converted by heat to the stable red pigment nitric oxide myo-hæmochromogen. The source of the nitric oxide is the nitrite present in the curing solution or pickle. Usually, the pickle consists of saturated salt solution containing sodium nitrate and sugar in which the meat is immersed, the solution sometimes being pumped into the meat to speed up diffusion. To inhibit putrefaction processes, the curing is carried out at a low temperature—about 5·5 deg. C. (42 deg. F.). Salt-tolerant bacteria developing in the pickle convert the nitrate to nitrite, the organisms concerned being of widely divergent types.

The elimination of sodium nitrate and the direct addition

of nitrite to the cure has been advocated but it would appear from recent investigations that this may have disadvantages from the preservative point of view, especially where canned cured meats are concerned. Jensen and Hess (1941), who review some of the earlier literature on the subject, found that nitrate in meat has an inhibitory action on putrefactive bacteria, 0·5 per cent. preventing the germination of *Clostridium sporogenes* spores except where contamination is unduly heavy. Their experiments indicated that nitrate in the amount normally present in nitrate-cured meat may cause a reduction in the heat resistance of putrefactive spoilage organisms. In stressing the value of nitrates in cured meats they remark that nitrite is to a large extent destroyed when heated with meat because it reacts with protein. Yesair and Cameron (1942) investigated the effect of curing salts on the growth and heat resistance of *Clostridium botulinum*. They showed that in meat-infusion agar more than 70 per cent. reduction in spore germination was produced by 0·1 per cent. sodium nitrate, 0·005 per cent. sodium nitrite or 2 per cent. salt. They suggest that the quantities used in commercial practice may result in complete inhibition. They also demonstrated an apparent reduction in the heat resistance of *Clostridium botulinum* when heated in cured meat but this effect was found to be due to the growth-inhibiting action of the curing salts. When heat-treated cured meats were cultured so that the inhibitory salts were diluted by the culture medium, the heat resistance proved to be unaffected. In phosphate buffer at *p*H 7·0, however, salt, sodium nitrate, and a mixture of the two appeared to cause a reduction in heat resistance at a temperature below 230 deg. F. (110 deg. C.). There was no appreciable effect in the range 230–235 deg. F. (110–112·7 deg. C.).

Smoking

The process of smoking meat and fish is carried out after brining by exposing the products to the smoke of smouldering

wood. In addition to imparting flavour the process has some preservative action which is ascribed to the absorption of bactericidal substances from the smoke. The chief of these appears to be formaldehyde. Except in very heavily smoked materials, however, the amount of formaldehyde present is small and it would seem probable that the preservative effect resulting from smoking is as much due to the presence of salt and the dehydration which occurs during the process as to the effects of the smoke itself. What little literature is available on the subject has recently been reviewed by Jones (1942), who gives detailed information on methods of smoking.

Fermentation

Vegetable products used in the manufacture of pickles are preserved by brining and fermentation. The vegetables are placed in brine, usually of about 5–10 per cent. concentration, and allowed to undergo spontaneous lactic fermentation. The salt restricts the activity of undesirable organisms but permits the development of the lactic group and other organisms which convert the natural sugars in the vegetable to lactic acid. In a report on cucumber fermentation, Etchells (1941) stresses the importance of yeast activity in the fermentation process. In addition to lactic acid, which is produced in sufficient quantity to exert a preservative effect, small amounts of alcohol and acetic and propionic acids are produced. The fermentation proceeds best at a temperature of about 25 deg. C. (77 deg. F.) and is normally complete in a few weeks, when the vegetables should be firm and translucent. The final acidity is usually in the region of 1 per cent. The fermentation may be speeded up by using low strength brines. Jones (1940) states that low salt brines (about 5 per cent.) favour the rapid formation of relatively high titratable acidity and low *p*H in cucumber fermentation. Increasing concentrations give slower acid formation with lower total acidity and higher brine *p*H.

When the vegetables are fully fermented it is customary to increase the salt strength to at least 15 per cent. to aid keeping quality. For successful storage it is essential to exclude air to prevent the development of film-forming fungi. These organisms oxidise the acid formed during the fermentation and thus permit growth of spoilage organisms which may cause softening and discolouration of the vegetables. Cruess (1938) recommends the use of a thin layer of slab oil to prevent film formation. His book contains extensive information on the fermentation of vegetables.

Filtration

The mechanical removal of micro-organisms by ultra-filtration is known as the " cold process " and is applied in the treatment of fruit juices, beers and wines. It is, of course, applicable only for clear, liquid products. The Seitz process which makes use of the special Seitz " E.K." filtering films is extensively used for sterilising liquids. The material is first given a preliminary clarification and then forced through a special filter press. In construction the press is similar to the common filter-press, the filtering agent consisting of films or pads of specially prepared asbestos and cellulose, the pores of which are sufficiently fine to impede the passage of micro-organisms. Unless preliminary clarification is carried out the pores rapidly become blocked. Before use, the assembled filter requires to be sterilised. This is usually accomplished by blowing steam under pressure through the filter for some 10–20 minutes. The sterile product issuing from the press may be filled aseptically into containers sterilised by steam or sulphur dioxide solution. The filtering films cannot be cleaned and, once used, are discarded.

REFERENCES

BARTON-WRIGHT, E. C., and TOMKINS, R. G. 1940. *Cereal Chem.*, **17** 332.

BAUMGARTNER, J. G. 1937. *Food Res.*, **2**, 321.

BERRY, J. A., and MAGOON, C. A. 1934. *Phytopath*, **24**, 780.
BUCHANAN, R. E., and FULMER, E. I. 1930. "Physiology and Biochemistry of Bacteria," Vol. 2. Baillière, Tindall and Cox, London.
BURGVITS, G. K. 1933. *Bull. State Inst. Agric. Microbiol., U.S.S.R.*, **5**, 166.
CALLOW, E. H. 1929. Food Invest. Bd., Dept. Sci. Ind. Res., London. Ann. Rept. 72.
CLOAKE, P. C. 1923. Food Invest. Bd., Dept. Sci. Ind. Res., London. Spec. Rept. No. 18.
CORRAN, J. W., and EDGAR, S. H. 1933. *J. Soc. Chem. Ind.*, **52**, 149T.
CRUESS, W. V. 1932. *Ind. Eng. Chem.*, **24**, 648.
CRUESS, W. V. 1938. "Commercial Fruit and Vegetable Products." McGraw-Hill Book Co., Inc., N.Y.
ERICKSON, F. J., and FABIAN, F. W. 1942. *Food. Res.*, **7**, 68.
ETCHELLS, J. L. 1941. *Food Res.*, **6**, 95.
FABIAN, F. W., and QUINET, R. I. 1928. *Mich. Agric. Exp. Sta. Tech. Bull.*, No. 92.
FABIAN, F. W., and WADSWORTH, C. K. 1939. *Food Res.*, **4**, 511.
FABIAN, F. W., KREHL, C. F., and LITTLE, N. W. 1939. *Food Res.*, **4**, 269.
GALLOWAY, L. D. 1935. *J. Textile Inst.*, **26**, 123T.
HAINES, R. B. 1934. *J. Hyg.*, **34**, 277.
HAINES, R. B. 1934a. Food Invest. Bd., Dept. Sci. Ind. Res., London. Ann. Rept. 44.
HARRISON, F. C., and KENNEDY, M. E. 1922. Rept. 11, Honorary Advisory Council Sci. Ind. Res., Canada.
HESS, E. 1934. *Contr. Canadian Biol. Fish.*, **8**, 491.
HOFFMAN, C., SCHWEITZER, T. R., and DALBY, G. 1939. *Food Res.*, **4**, 539.
JENSEN, L. B., and HESS, W. R. 1941. *Food Manuf.*, **16**, 157.
JONES, I. D. 1940. *Ind. Eng. Chem.*, **32**, 858.
JONES, O. 1942. *Food*, **11**, 191.
JOSLYN, M. A., and CRUESS, W. V. 1929. *Hilgardia. Calif. Agric. Exp. Sta.*, **4**, 201.
KARAFFA-KORBUTT, K. V. 1912. *Z. Hyg.*, **71**, 162.
LABRIE, A., and GIBBONS, N. E. 1937. *J. Biol. Bd., Canada*, **3**, 439.
LEA, C. H. 1931. *J. Soc. Chem. Ind.*, **50**, 207T.
LEVINE, A. S., and FELLERS, C. R. 1940. *J. Bact.*, **39**, 499.
LEVINE, A. S., and FELLERS, C. R. 1940a. *J. Bact.*, **40**, 255.
GARRARD, E. H., and LOCHHEAD, A. G. 1939. *Canad. J. Res.* Sect. D 17 (2); 45.
MCCLESKEY, C. S., and CHRISTOPHER, W. N. 1941. *J. Bact.*, **41**, 98 (Abst.).
MCCULLOCH, E. C. 1936. "Disinfection and Sterilisation." Henry Kimpton, London.
MCFARLANE, V. H. 1941. *Food Res.*, **6**, 481.
MORAN, T. 1938. *Food Res.*, **3**, 149.
MRAK, E. M., and BONAR, L. 1939. *Zentralbl. Bakt.*, 11 Abt., **100** (14/17); 289.

NUNHEIMER, T. D., and FABIAN, F. W. 1940. *Amer. J. Pub. Health*, **30**, 1040.
OSLEN, C. J., and MADY, H. 1940. *J. Dairy. Sci.*, **23**, 509 (Abstr.).
PEDERSON, C. S., and BREED, R. S. 1926. *N.Y. Agric. Expt. Sta. Bull.*, 538.
POUNCY, A. E., and SUMMERS, B. C. L. 1939. *J. Soc. Chem. Ind.*, **58**, 162.
ROBERTSON, M. E. 1931. *J. Hyg.*, **31**, 84.
SCHOOP, G. 1929. *Deutsche Tierarztl. Wochenschr.*, **37**, 753.
SMART, H. F. 1934. *Phytopath.*, **24**, 1319.
STEWART, M. M. 1934. *J. Soc. Chem. Ind.*, **53**, 273T.
STUART, L. S. 1938. *Food Res.*, **3**, 417.
STUART, L. S. 1940. *J. Agric. Res.*, **61**, 267.
STUART, L. S., and JAMES, L. H. 1938. *J. Bact.*, **35**, 381.
TANNER, F. W. 1932. "Microbiology of Foods." Twin City Printing Co., Champaign, Illinois.
TOPLEY, W. W. C., and WILSON, G. S. 1936. "Principles of Bacteriology and Immunity." Arnold & Co., London.
URBAIN, W. M., and JENSEN, L. B. 1940. *Food Res.*, **5**, 593.
WILSON, H. F. 1934. *Food Manuf.*, **9**, 162 and 215.
YESAIR, J., and CAMERON, E. J. 1942. *Canner* (Convention No.), **94**, 89.

CHAPTER III

OUTLINE OF CANNING OPERATIONS

Can Manufacture

For those who have an interest in the bacteriological condition of canned foods, a working knowledge of the fundamentals of can seam formation is essential since the maintenance of sterility is dependent on the efficiency of the can seaming operations.

The earlier types of metal containers were made exclusively by hand. All seams were jointed by solder and the food was filled into the can through a small hole in the end which was afterwards covered by a disc or cap which was soldered in place. The inherent disadvantages with these "hole and cap" containers included the difficulties of packing large pieces of food through the relatively small hole and of efficient cleansing of the can before filling. Out of these disadvantages and the need for increased production arose by degrees the modern "open-top" or sanitary can which consists of a cylindrical body with a soldered lock seam and unsoldered double-seamed ends. Except for a few products, the "open-top" can has to a very large extent superseded other types of containers.

The high speed of modern production and the function which the can has to fulfil in hermetically sealing the food necessitates that all the processes concerned in can fabrication and seaming are precision operations. Briefly, typical operations involved are as follows :—

Body Blanks. The blanks for the can bodies are cut from sheet tinplate on a machine known as a Gang Slitter. The blanks must be cut absolutely square and the sheet should be cut so that the "grain" of the plate runs in the direction of the length of the blank. For certain foods such as some

fruits, vegetables and meat products, the tinplate needs to be protected by a film of lacquer which may be applied to the sheets of tinplate before they are cut and made into cans. With this method, however, the lacquer film generally sustains damage during the fabrication of the can, with the result that bare metal is exposed to any attack by food placed in the can. A method of lacquering designed to obviate such damage consists of flushing the can with lacquer after it is made. The application of a lacquer film to cans by electro-deposition has been advocated by Clayton (1936).

Body Forming. The blanks are formed into cylindrical bodies by the Body Maker which automatically performs several successive operations. First, the corners of each blank are notched so that when the lock seam is formed the ends of the seam comprise only two thicknesses of metal. The significance of this notching operation becomes apparent in the formation of the double seams. After notching, the blank is edged, that is a hook is formed on each of the two shorter edges of the blank by bending one edge downwards and the other edge upwards. The latter hook then receives an application of flux, after which the blank is formed into a cylindrical body by being wrapped by means of two " wings " round an expanding cylinder or horn. The hooked edges of the blank engage each other and the horn expands so that the hooks lock tightly together. The locked hooks are then hammered flat against the horn. After a second application of flux to the outside of the lock seam the body is next carried over a revolving solder roll. The solder roll rotates in a bath of molten solder and is aligned in such a manner that solder is applied to the lock seam as the can body passes along it. Immediately after leaving the solder roll excess solder is removed from the seam by a revolving " brush." Before the can body finally leaves the Body Maker it receives a blast of cold air to cool and solidify the solder in the lock seam.

Flexing. It is important for the success of subsequent operations that the can body be perfectly rounded and the flexing machine is designed to iron out any flat surfaces or crimps by spinning the body between rubber rollers.

Flanging. The can bodies are next fed to the Flanger, where they are centred between two flanging punches

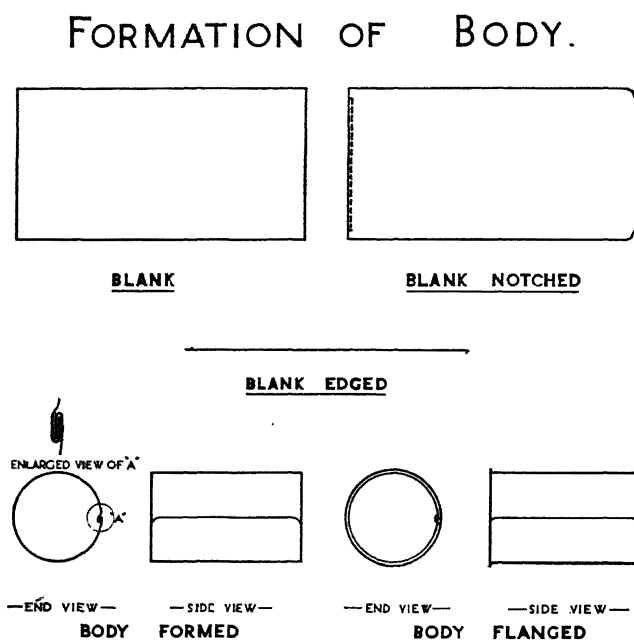


FIG. 1.

which fit into either end of the can. These punches then move slightly towards each other and force the edges of the body outwards, thus simultaneously forming flanges on both ends of the can body.

Manufacture of Can Ends. The strips from which the ends are stamped out are cut from sheet tinplate by the Scroll Shears machine. From the point of view of wastage of

tinplate it is more economical to stamp out the ends in a staggered or scroll pattern than in straight, parallel rows and the sheets are therefore cut into scrolled strips. The ends are stamped out from the scrolled strips by automatic

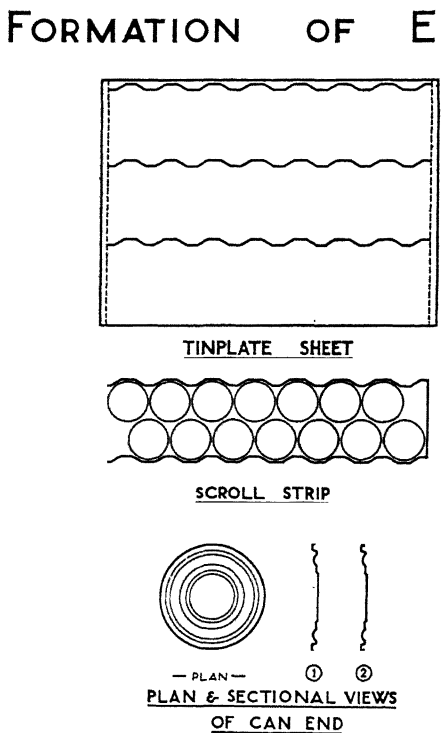


FIG. 2.

presses, the concentric rings, termed expansion rings, which are formed on the ends by the press being designed to permit ready bulging of the ends during heat-processing and thus to relieve strain on the seams of the can. Another advantage arising out of the use of these rings is the readiness with which the ends bulge in case of spoilage.

In addition, their springiness is an aid to good double-seam formation.

After the rim of the ends has been curled inwards by the Curling Machine, the inside of the curl is lined with a sealing compound which may be rubber solution, rubber latex or some other material with similar resilient properties. The

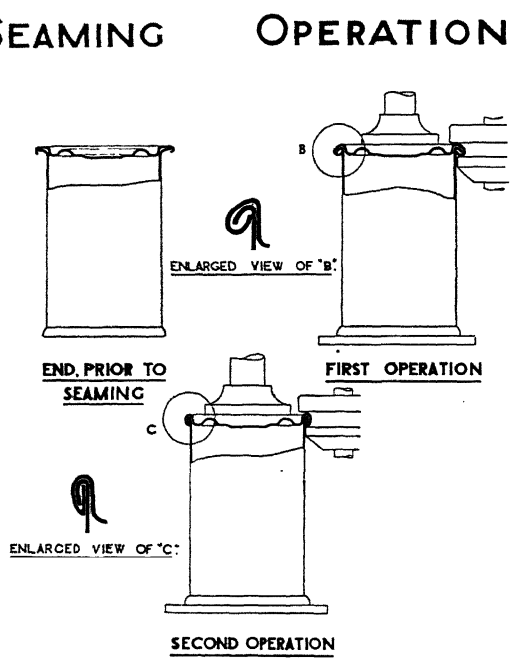


FIG. 3.

sealing compound, which is dried by passing the ends through an oven, acts as a gasket and ensures an airtight seal in the finished seam by filling in the small irregularities between the metal surfaces of the seam.

Double Seaming. The can ends are attached to the body by a Double-Seamer machine. Although there are a number of different seaming machines, fundamentally they consist

of a base plate or table on which stands the can to be closed, a chuck which fits firmly into the countersink of the can end and a pair of seaming rolls or grooved wheels which operate in succession. As the can body is delivered to the Double Seamer it is automatically covered by an end, the curl of which fits over the flange of the body. The body with the end in place is carried to the base plate, which by lifting automatically, causes the chuck to fit into the end of the can. The first of the seaming rolls then comes into operation and by pressing tightly and revolving at high speed round the curled rim of the end, rolls or tucks it under the flange of the body. The second wheel, which has a more shallow groove than the first, then comes into operation and tightly flattens together the hooked edges of the end and the body against the body of the can. The finished double seam, which should conform to certain standards of measurement, consists of five thicknesses of metal except where it coincides with the body seam where seven thicknesses are present. If the body blanks were lock-seamed right up to the edges, the double seam would, at the point where it meets the lock seam, comprise eleven thicknesses of metal. By notching the blanks four thicknesses of metal are eliminated. The various processes involved in can manufacture are shown diagrammatically in Figs. 1, 2 and 3.

Preparation of Food for Canning

The canning of food involves a series of operations, each of which has an important bearing on the quality of the final product. Especially does this apply to the selection of raw materials which must be sound and clean. The use of any material showing obvious signs of spoilage will inevitably result in some deterioration of quality of the product and scrupulous cleanliness is essential. Soil is frequently a prolific source of spoilage organisms and even after thorough washing, soil-contaminated products such as vegetables will still carry a large number of spoilage

organisms. If vegetables are packed with adherent soil the number of organisms entering the can will be enormously increased with the result that the heat-processing conditions may be inadequate to deal with them (see section on the principles of heat-processing).

Having due regard to efficiency, the canning process should be completed as rapidly as possible with the minimum of delay between preparation of the food and processing. Particularly with moist materials such as meat pastes for example, a delay or two or three hours is sufficient to permit the development of rapidly growing organisms which may cause spoilage due to gas production or souring.

Many products such as vegetables, fruit and meat are scalded or blanched in hot water or steam before canning. This has for its objects the cleansing of the product, shrinkage to permit adequate filling of the can and the removal of respiratory gases from the cellular tissue. Except where there is some reduction of infection by washing, the effect of blanching on heat-resistant spoilage bacteria is negligible.

The hygienic condition of plant and equipment is a factor of major importance. Instances of spoilage are known where the product was inoculated by heavily infected material which had remained stagnant in pipe-line "dead ends." Over-night development of organisms in improperly cleaned plant may also result in heavy contamination of material canned the next day.

Exhausting

An essential operation in the canning process is the removal of air from the can before it is closed. This procedure is necessary for the following reasons :—

1. Minimisation of strain on the can through expansion of air during heat-processing.

2. Removal of oxygen which accelerates internal corrosion of the can.

3. Creation of a vacuum when the can is cooled. Cans with bulging ends being regarded as unsound, it is necessary to ensure that the can ends remain flat or slightly concave throughout moderate changes of storage temperature or barometric pressure.

In commercial practice two procedures are adopted for the removal of air from the can. In the heat-exhaust method the contents of the can are heated immediately before sealing, so causing the air in the headspace to be displaced by water vapour with the result that a vacuum is formed when the can is cooled to a temperature below that at which it was sealed. Other factors being equal, the vacuum so formed increases with the closing temperature. During heat-exhausting the can contents expand and the contraction which takes place on cooling also contributes towards the formation of the vacuum. In addition to the temperature at which the can is sealed, the volume of the headspace in the can is an important factor in vacuum formation. If the headspace is too great when the can is sealed a relatively large amount of air will be trapped in the can with the result that a low vacuum will be formed on cooling. On the other hand, overfilling is to be avoided if the can ends are to remain flat and straining during retorting is not to occur. The usual custom is to fill within $\frac{1}{4}$ to $\frac{3}{8}$ in. of the top of the can. An incidental advantage which accrues from heat-exhausting and which is often of great practical importance is that it enables a shorter heat-process time to be applied due to the fact that the cans enter the retort partially heated. Heat-exhausting can be applied in two ways; the contents may be heated immediately before filling into the can which is then quickly sealed or they may be filled cold into the can, which is then passed through a steam-heated chamber before sealing. In the latter case the cans may be exhausted with clinched ends, that is, ends loosely attached so that escape of air is permitted, seaming being completed when the cans leave

the exhauster. The heat-exhausting process known as "brogging" has been almost completely replaced by the more modern methods. In "brogging," the cold-filled and sealed can is heated in a retort until the contents are quite hot. The can end is then pierced to allow escape of steam and air and the hole immediately closed by a blob of solder. Heat-processing is then carried out in the normal way. An alternative method is to exhaust the can with an open brog-hole, which is then sealed and the can processed in the normal way. It was once the custom of unscrupulous or ignorant packers to "brog" and re-process spoiled cans and consequently cans with soldered "brog-holes" have come to be regarded with suspicion in some quarters.

In the other exhausting procedure, which is referred to as vacuumising or mechanical exhaust, the cold material is filled into the can, which is then closed in a vacuum closing machine, the can being subjected to a high vacuum during the seaming operation. For canned meat pastes or other products in which air is occluded during their preparation, heat-exhausting is desirable. The heating method is also desirable for unblanched vegetables or fruits, the cells of which contain respiratory gases, which are not readily removed by vacuumising.

Processing

After exhausting and closing the cans are heated for an accurately predetermined time and temperature in an atmosphere of saturated steam or in steam-heated water. This operation, which is usually referred to as "processing," may be regarded as the crux of the whole canning procedure, as the keeping properties and to some extent the quality of the food depend upon the use of correct processing technique. Processing is mainly carried out in batch retorts or pressure cookers. A comparatively modern development is the use of continuous, agitating pressure cookers in which the steam pressure can be maintained while the cans are fed

and removed after processing through specially constructed ports. Such continuous cookers have considerable advantages in reducing the over-all time necessary for the processing operation but they are not yet in general use.

The sterilising action of steam depends largely upon the transfer of its heat of vaporisation to the surface of the cans on which it condenses. Dry or superheated steam condenses less readily and is therefore less efficient than saturated steam in transferring heat. The complete elimination of air from the retort is a vitally important factor in processing technique and retorts or cookers should be so constructed that removal of air is facilitated. Modern retorts are constructed with this in view. The steam should enter the retort at the bottom, and be so distributed as to ensure that air is blown out through the valves or vents at the top ; an adequate trap for condensate is necessary. Immersion of cans in water is to be avoided since the cans must be completely surrounded by steam if it is to exert its maximum heating efficiency.

One effect resulting from the presence of air in the retort is that for any given pressure the temperature is lower than that obtained with steam alone. The total pressure of a mixture of air and steam is equal to the sum of the partial pressures but the temperature of the mixture will correspond to the pressure of the steam only. The effects of air in lowering the efficiency of steam sterilisation were investigated by Hoyt, Chaney and Cavell (1938). Their conclusions were :—

1. Air reduces the retort temperature and this is an uneven phenomenon because air, being heavier than steam, tends to stratify below the steam.

2. A mixture of air and steam at any temperature is not as efficient as saturated steam at the same temperature.

3. Air in the retort cuts down the penetration of the steam.

From these considerations it will be clear that sole reliance in pressure-processing should not be placed on

pressure gauges ; processing should be controlled by pressure and temperature readings between which there must be complete agreement. Agreement will not be obtained unless all air has been eliminated from the retort. The accuracy of pressure gauges and thermometers or temperature recorders is obviously important and these should be checked periodically.

A permanent record of each processing cycle is regarded as essential in modern cannery control. Such a record is obtained by means of automatic temperature recorders, the process temperature being traced on a revolving chart marked with time-temperature scales. Usually, the recorder mechanism consists of a temperature-sensitive bulb containing a volatile liquid. The bulb is connected by capillary tubing to one end of a hollow metal coil, the other end of which is fitted with a pen arm. The temperature sensitive bulb is fitted through the wall of the retort. As the temperature of the retort rises, increase in the vapour pressure of the liquid in the bulb results in a change in tension of the coil and a corresponding movement of the pen arm on the chart. Latest practice calls for automatic control of the processing operation. Full details of automatic recording and controlling instruments is to be found in the catalogues issued by the manufacturers.

Cooling

At the conclusion of the heating process, the cans are cooled as rapidly as is consistent with avoidance of seam strain in order to prevent over-cooking of the foods. This cooling operation is very important and if mismanaged may be the cause of considerable trouble and loss. During heat-processing the internal pressure of the can increases due to the expansion of the residual air, the increase in the vapour pressure of the water in the product and the expansion of the can contents. At the conclusion of the actual heating period, the internal pressure of the can is at its maximum

but is partially balanced by the steam pressure in the retort. Provided that the can is closed under conditions giving a minimum amount of residual air in the can the difference between the internal pressure in the can and the external steam pressure is insufficient to cause serious straining during the heating period. If, however, the steam pressure in the retort is permitted to drop suddenly, the can may be permanently distorted or the ends may be forced out to such an extent that severe straining of the seams results with consequent risk of leakage.

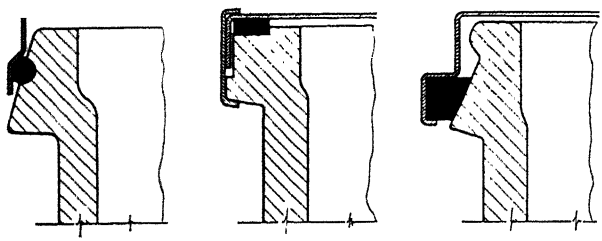
In order to obviate such straining, pressure cooling is often applied, and indeed is essential for the larger sized cans. Fundamentally, this operation consists of maintaining the retort pressure during the cooling period by replacing the steam with compressed air, the air pressure being maintained while the retort is flooded with cold water. A simplified method of pressure cooling has been outlined by the Research Department of the Metal Box Co. Ltd. (1941). Where pressure cooling in the retort is not applied, the retort pressure is allowed to drop slowly to atmospheric pressure and the cans are then removed and sprayed with cold water or passed through a water tank. The cans should be permitted to retain sufficient heat to ensure rapid drying and thus obviate rusting.

Glass Containers

A wide variety of foods are packed in hermetically sealed glass containers fitted with metal closures and although such containers require treatment differing slightly from that used for food packed in cans, the general principles involved are the same in both cases.

Types of Closures. The metal caps are usually of lacquered tin-plate. A paper liner or disc is sometimes used to prevent contact of the food with the metal cap, thus avoiding any tendency for discolouration or corrosion to occur. The basis of all closures for air-tight glass containers is that the

metal cap is firmly held against a rubber gasket which seats on or round the rim of the glass, the various closures



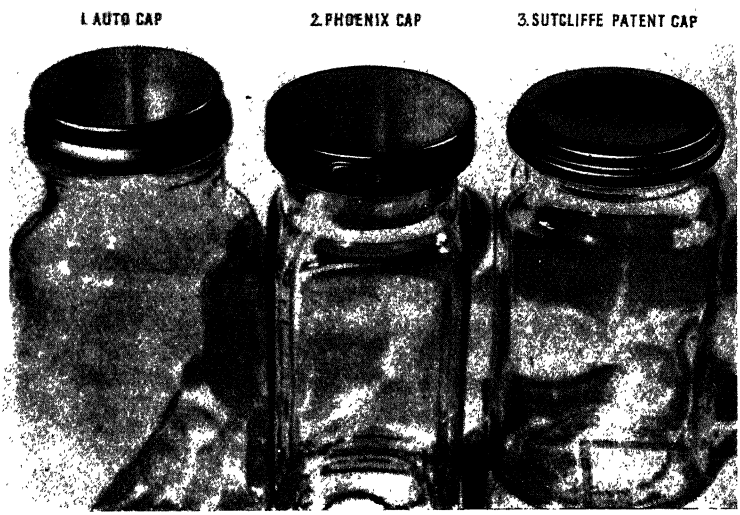


FIG. 4.

differing chiefly in the means by which the metal cap is held in place. The types of closure most frequently employed commercially are the Phœnix and the Automatic, both of which are illustrated in Fig. 4. Also shown, is the Sutcliffe

Patent closure, which is a recent development in Automatic-type closures for glass containers.

Phœnix. In this system the cap is clamped tightly against the rubber gasket by means of a metal band which is clinched on by machine, the lower edge of the band being tucked under the projecting rim of the glass and the upper edge turned over the rim of the cap Packs with this type of closure may be mechanically or heat-exhausted or sealed at atmospheric pressure.

Automatic. This is a " one piece " closure. The cap is firmly held against the rubber gasket by means of a vacuum in the jar. Containers with automatic closures may be sealed under vacuum or the method of automatic exhaust during processing may be used. In the latter case the cap is firmly held against the gasket by means of spring clips and during processing a valve effect takes place, the internal pressure due to air expansion and steam causing the cap to lift and thus permit escape of steam and air. As the jar cools a vacuum is formed which firmly holds the cap in place and the clips are removed.

Processing. Because of the risk of fracture of the glass, lower temperatures and longer exposures are generally used for processing glass-packed foods than is customary for canned products. For the same reason, the heating and cooling of glasses must also be carried out slowly. The modern method of processing glass containers entails heating in steam-heated water on which air pressure is super-imposed. The containers are placed in the retort and covered with cold water. Air is admitted to give a pressure of about 10 lb. per square inch and the water is then heated to the desired temperature by steam. Throughout the process the excess air pressure in the retort is maintained. At the conclusion of the heating period the steam is shut off and cooling is carried out by slowly admitting cold water to the retort, the caps being prevented from " blowing " off by the air pressure which is maintained during the whole of the cooling

period. This method is applicable to containers with Phœnix or vacuum-sealed Automatic closures. Alternatively, glass containers may be processed in steam and air-cooled but as stated above, Automatic closures so treated need to be fitted with clips.

REFERENCES

CLAYTON, W. 1936. B.P. 455,810.
HOYT, A., CHANEY, A. L., and CAVELL, K. 1938. *J. Bact.*, **36**, 649.
METAL BOX CO. RES. DEPT. 1941. *Food*. **10**, 150.

CHAPTER IV

PRINCIPAL SPOILAGE ORGANISMS IN CANNED FOODS

MICROBIAL spoilage in heat-preserved canned foods is due to the activity of micro-organisms which survive the heat-processing of the cans or those which gain access through leaks in the containers after processing. In under-processed canned foods, it is often possible from a knowledge of the processing conditions and the nature of the food, to predict the type of organism which is responsible for the spoilage. In "leaker" spoilage, however, the type of infection is usually unpredictable since the microbial condition of the cooling medium (water and air) from which the spoilage organisms are derived, may be extremely varied.

American workers have shown that the type of microbial spoilage occurring in under-processed packs is related to the acidity of the food. Bigelow and Cameron (1932) proposed a classification of canned foods, based on acidity as follows :—

Non-acid foods : *p*H value above 6·0.
Semi-acid foods : *p*H value between 4·5 and 6·0.
Acid foods : *p*H value below 4·5.

Cameron and Esty (1940) have suggested modifications to this classification. They recognise four acidity groups, to each of which they have assigned special spoilage relationships :—

Group 1. Low-acid (*p*H 5·0 and higher) : Meat products, marine products, milk and certain vegetables.

Group 2. Medium-acid (*p*H 5·0 to 4·5). Meat and vegetable mixtures, specialities such as spaghetti, soups and sauces.

Group 3. Acid (*p*H 4·5 to 3·7) : Tomatoes, pears, figs, pineapple and other fruits.

Group 4. High-acid (*p*H 3·7 and below) : Pickles, grapefruit, citrus juices and rhubarb.

In so far as canned products in this country are concerned the *p*H values given by Cameron and Esty appear to be low in certain instances, as for example, meat and vegetable mixtures and most of the soup varieties which are usually above *p*H 5·0. It is accepted as a general principle in the canning industry that for foods with a *p*H value below 4·5, pressure-processing is unnecessary because of the inability of the heat-resistant food poisoning organism *Clostridium botulinum* to develop below this level. In Cameron and Esty's classification this demarcation is still recognised ; the sub-dividing of the acid group into " acid " and " high-acid " was necessitated by the incrimination of acid-tolerant, spore-forming bacteria as the spoilage agents in certain canned fruit and tomato products with *p*H values between 4·5 and 3·7. Spoilage of this type does not appear to have been experienced in products packed in this country. In the following brief review, the principal spoilage organisms are treated under two main headings according to their significance for low and medium-acid foods and acid foods.

Low-acid and Medium-acid Products

Thermophiles. As causes of spoilage in under-processed canned foods, the incidence of the various organisms which affect the different groups of food is generally related, as would be expected, to their heat resistance. It is not surprising, therefore, that the organisms most frequently encountered in heat-treated low and medium-acid foods are the thermophiles since it is characteristic of this group that the spores of many species exhibit a phenomenal resistance to destruction by heat. Bigelow and Esty (1920) observed that certain strains survived continuous boiling in corn juice at *p*H 6·1 for 24 hours. Donk (1920) reported the thermal death-time of a " flat-sour " type as 11 minutes at 120 deg. C. (248 deg. F.) at *p*H 6·1 and a " flat-sour "

organism studied by Williams, Merrill and Cameron (1937) survived 35 minutes at 120 deg. C. (248 deg. F.) in buffer (pH 6·95).

The thermophilic bacteria have an optimum growth temperature of about 55 deg. C. (131 deg. F.). Three main groups are recognised :—

1. " *Flat-Sour* " *Group.* The facultative anaerobic group of " flat-sour " organisms are so called because they attack carbohydrates with resultant acid but no gas formation ; in canned foods spoiled by these organisms there is consequently no depreciation of vacuum, the ends of the can remaining flat or concave. The " flat-sour " types are economically the most important of the thermophiles as spoilage agents for the reasons that their incidence is higher and for some of the species the temperature range for growth is wider than that of the other groups. Growth at 37 deg. C. (98·6 deg. F.) is common and some species develop slowly at temperatures around 25 deg. C. (77 deg. F.). Products infected with these organisms and stored for long periods at such temperatures may show souring. Cream-type soups and similar products in which quality considerations necessitate a minimum of heat-processing are particularly liable to suffer spoilage of this type. The species developing below 40 deg. C. (104 deg. F.) are termed facultative thermophiles in contrast to the obligate thermophilic species which fail to grow below this temperature. Bigelow and Cameron (1932) have observed that the obligate types are generally more heat-resistant than the facultative types.

The type species of the " flat-sour " group is *Bacillus stearothermophilus*, which was described by Donk (1920). This organism possesses the following characteristics :—

Spores : Ovoid, 1·0 by 1·5 microns, terminal.

Sporangia : Swollen, clavate, not in chains.

Rods : 0·8 by 3·5 microns, usually single, sometimes in pairs or chains of 3–4 cells, with rounded ends. Non-motile. Gram-negative.

Gelatin : No liquefaction.
Agar colonies : Circular, flat, entire, smooth. An opaque colony in centre surrounded by several concentric zones.
Agar slant : Growth filiform to beaded, not spreading, translucent, dirty white, butyrous.
Broth : Turbid. No surface growth. Abundant sediment.
Potato : No growth.
Litmus milk : Acidified, not coagulated, casein digested. Litmus reduced.
Indol not formed.
Nitrites not produced from nitrates.
Starch is hydrolysed.
No gas from dextrose, lactose and sucrose.
Thermophilic, optimum temperature 50 deg. C. Maximum 76 deg. C. (178·8 deg. F.). Minimum 45 deg. C. (113 deg. F.).
Aerobic, facultative anaerobic.
Source : Isolated from samples of spoiled canned corn and string beans.
Habitat : Probably found in soil and dust.

Although most low and medium-acid foods may be affected, products containing sugar or starch are particularly liable to undergo severe spoilage by the " flat-sour " organisms.

2. *Anaerobes not producing* H_2S. Next in importance is the group of strictly anaerobic thermophiles, the type species of which was named *Clostridium thermosaccharolyticum* by McClung (1935). Its general characteristics are :—

Rods : 0·4–0·7 by 3·5–7·5 microns, slender, granulated, occurring singly and in pairs, not chains. Motile with peritrichous flagella.
Spores spherical, terminal, swelling rods. Gram-negative.
Gelatin : Not liquefied.
Pea-infusion agar surface colonies : Granular, greyish white, raised centre with feathery edges.
Deep dextrose-tryptone agar colonies : Small, lenticular, smooth.

Liver-infusion broth over liver meat : Turbidity and gas.
Litmus milk : Litmus reduced. Acid and firm coagulum split with gas. Clot not digested.
Indol not formed.
Nitrites not produced from nitrates.
Cellulose not fermented.
Acid and gas from arabinose, levulose, galactose, dextrose, mannose, xylose, cellobiose, lactose, sucrose, maltose, corn starch, trehalose, dextrin, glycogen, amygdalin, esculin, and salicin.
Coagulated albumen and blood serum not liquefied.
Brain or meat medium not digested or blackened.
Non-pathogenic on feeding to white rats or injection into rabbits.
Optimum temperature 55–62 deg. C. (131–143·6 deg. F.).
Thermophilic.
Anaerobic.
Isolated from hard " swell " of canned goods and from soil.

The organisms of this group are markedly saccharolytic, producing large quantities of gas, chiefly carbon dioxide and hydrogen, from carbohydrates and consequently they cause spoilage of the swell or gaseous type in which the ends of affected cans are distended. Cameron and Esty (*loc. cit.*) state that in products of moderate acidity (*p*H 5·0–4·5) these organisms are of greater significance as spoilage agents than the " flat sour " types.

The growth temperature range of the saccharolytic anaerobes is generally narrower than that of the " flat sour " species. Of the cultures examined by McClung (1935) only one showed slight growth after 30 days incubation at 30 deg. C. (86 deg. F.). The members of this group are, therefore, not responsible for appreciable losses in this country since storage temperatures are not normally favourable for their growth. They are, however, a considerable source of trouble in products exported to tropical countries.

3. *Anaerobes producing* H_2S. The third group is characterised by the production of hydrogen sulphide and is responsible for the so-called " sulphur stinker " spoilage in canned foods. The type species is *Clostridium nigrificans* (Werkman and Weaver, 1927). This organism is only feebly saccharolytic, its general characteristics being as follows :—

Rods : 0·2–0·5 by 3·0–6·0 microns. Rounded ends. Motile. Spores oval, sub-terminal, slightly swelling rods. Gram-positive.

Gelatin : Not liquefied.

Deep agar colonies : Show blackening of medium around colonies. Black increased by adding 0·1 per cent. of ferric chloride to medium.

Milk : Not recorded.

Indol not formed.

Nitrites not produced from nitrates.

Dextrose not fermented.

Coagulated albumen or blood serum not liquefied.

Brain medium blackened but not digested.

Hydrogen sulphide produced from cystine.

Non-pathogenic.

Optimum temperature 55 deg. C. (131 deg. F.).

Anaerobic.

Distinctive character. Black colonies in agar media.

Isolated from canned corn showing " sulphur stinker " spoilage, and occasionally soil and manure.

Habitat : Presumably soil.

Cans infected with this organism usually remain flat due to the fact that the hydrogen sulphide is soluble in the contents which become black as the result of interaction between the sulphur compound and the iron of the container. Spoilage of this type is comparatively rare.

In addition to the types noted above, other species of aerobic, spore-forming thermophiles may be isolated from canned foods. These organisms are, however, not saccharo-

lytic and do not produce gas and they do not appear to be associated with any spoilage processes in canned foods.

Since the heat-resistance of the thermophiles is such that thermal processing sufficient to ensure their destruction often results in deterioration in the quality of the food, their control chiefly consists in the elimination or reduction of infection and the rapid cooling and storage of infected products at low temperatures.

Because of their importance in canned food spoilage there have been numerous investigations into the sources of thermophilic organisms. These researches have mainly been carried out by workers in America where higher prevailing temperatures cause these organisms to have a greater significance as spoilage agents in canned foods than they normally possess for products packed in this country. Among the materials in which thermophiles have been found to be present in significant numbers are soil, particularly manured soil, sugar, starches of various kinds, flour, milk powder and gelatin. As vehicles for infection with these organisms, sugar and starches were discovered to be exceedingly important, with the result that the National Canners' Association Research Laboratories have set up standards limiting the thermophilic contamination of these ingredients for canning purposes. These standards and the methods of testing the materials are given later. A report by Barton (1938) on some English raw materials indicated heavy thermophilic infection in many cases. Sugar, syrups, arrowroot, cornflour, riceflour and mushrooms were among the materials tested with a high thermophilic spore content.

Spore-Forming Mesophilic Anaerobes. Next in importance to the thermophilic group as spoilage agents for canned foods are the mesophilic, sporing anaerobes. The optimum growth temperature of most species in this group is about 37 deg. C. (98·6 deg. F.) but many develop at 20 deg. C. (68 deg. F.) or lower and a few can grow at 50 deg. C. (122 deg. F.) and above. Although classification of these organ-

isms is primarily based on morphological and other characteristics, a rough grouping is possible on the basis of the relative ability of the various species to attack proteins and carbohydrates. Thus, there is a proteolytic or putrefactive group which includes such species as *Clostridium botulinum*, *Clostridium sporogenes* and *Clostridium bifermentans*, and a saccharolytic group which is represented by *Clostridium butyricum* or *Clostridium pasteurianum*, *Clostridium welchii* and other species.

The significance of the groups as spoilage agents is related to the type of food and general experience seems to be that for low-acid or medium-acid products the putrefactive group is the most important. Outstanding in this group is the pathogenic organism—*Clostridium botulinum*, which is discussed in the section on food poisoning. Destruction of the spores of this organism is generally accepted as the minimum standard of processing for low-acid and medium-acid canned foods. This species is not, however, as heat-resistant as some other members of the putrefactive group, strains resembling *Clostridium sporogenes* appearing to be the most resistant. An organism studied by Townsend, Esty and Baselt (1938) survived 35 minutes heating at 110 deg. C. (230 deg. F.) in phosphate buffer solution at *p*H 7·0. Baumgartner and Wallace (1936) isolated a strain closely related to *Clostridium sporogenes* which remained viable after 30 minutes heating at 115 deg. C. (239 deg. F.) in buffer (*p*H 7·0). Of the putrefactive anaerobes, organisms of the *Clostridium sporogenes* type appear to be most frequently involved in the spoilage of canned foods. Crossley (1941) observed that *Clostridium sporogenes* types were the most serious cause of spoilage in canned meat and fish products, and the spoilage of canned vegetables such as beans, peas, potatoes and tomato products of low acidity was principally caused by organisms of the *Clostridium butyricum* type. In an earlier report, Crossley (1938) stated that spoilage in meat and fish pastes was mainly due to

putrefactive anaerobes (*Clostridium sporogenes* and *Clostridium putrificum*) but noted that these organisms failed to develop in products with *p*H values below 6·0. According to Cameron and Esty (1940) putrefactive anaerobic species may show abnormal development resulting in non-gaseous spoilage in medium-acid products (*p*H 5–4·5).

Usually, the spoilage produced by the putrefactive anaerobes is of the gaseous type and is often accompanied by disintegration of the food and the production of characteristic foul-smelling compounds.

The spore-forming anaerobes are mainly derived from soil, which fact accounts for their fairly wide distribution in dust, milk, vegetables and other food materials. Some species are also commonly found in human and animal intestines. Due to their presence in animal excreta they are frequent contaminants of meat with the result that they have been prominently associated with the spoilage of canned meat products. These organisms, however, have also been responsible for losses in canned milk, cream, fish, vegetables and other products.

Spore-forming aerobes. The aerobic spore-forming mesophiles of the genus *Bacillus* are widely distributed in nature. Originating in soil and water, they are frequently present in dust, air and are found in practically all the raw materials used in food canning. For most members of the group, the optimum temperature for growth lies between 25 deg. and 37 deg. C. (77–98·6 deg. F.) but some species can develop at 50–55 deg. C. (122–131 deg. F). With regard to their oxygen relationship, some species are obligate aerobes but many are facultatively anaerobic.

It is sometimes stated in the literature that the heat-resistance of the members of this group is generally lower than that of the putrefactive anaerobes. Some aerobic spore-formers however, as for example the species which have been associated with the production of " off " flavours and " thinning " in canned cream, have been credited with

considerable resistance to heat destruction. McMaster (1932–1933) investigated an organism allied to *Bacillus cohœrens* which caused spoilage of canned cream and which survived 10 minutes heating at 115·5 deg. C. (239·9 deg. F.) in milk at *p*H 7·2 and Nichols (1940) has reported that many of the strains of aerobic spore-formers isolated from canned milk products withstood heating for 20 minutes at 120 deg. C. (248 deg. F.). Crossley (1938) found the heat resistance of the aerobic spore-formers to be about the same as that of the putrefactive anaerobic species.

The aerobic, mesophilic spore-formers are not very frequently incriminated in spoilage processes in canned foods and this is sometimes stated to be due to the depressant effect of vacuum on their growth and their relative inability to produce very marked changes in the food. It has been shown however, that although normally unable to produce gas from carbohydrate media, certain species may cause gaseous spoilage in sugar-nitrate cured canned meats (Jensen, Wood and Jansen, 1934). Experiments carried out by Jensen and Hess (1941) indicated that sodium nitrate was essential for gas production by the organisms studied by them. Michael and Tanner (1936) have observed that organisms of the aerobic, spore-forming type may produce gas in canned corn. In connection with influence of vacuum on the activity of organisms belonging to this group, Fellers (1927) has reported that the spoilage of canned fish products by aerobic spore-formers is not prevented by the reduced oxygen tension in evacuated cans, although the spoilage is less marked with cans which have a vacuum of from 8–10 in.

Yeasts, Moulds and Non-spore-forming Bacteria. Apart from spoilage arising through leakage of the container, these organisms of low heat-resistance have no significance in the spoilage of low-acid or medium-acid canned foods with the exception of sweetened condensed milk. This product is not heat processed, its keeping properties depending on its high sugar content. Discussing canned milk products,

Nichols (1936) states that spoilage of sweetened condensed milk may be due to gas-forming yeasts. Mould species, notably *Aspergillus repens*, are associated with the formation of " buttons " which are formed on the surface of the product. The " buttons " are reddish-brown in colour and are about $\frac{1}{4}$–$\frac{3}{4}$ in. in diameter. Lipase-secreting bacteria such as *Pseudomonas fluorescens* may cause rancidity as well as yeasts or moulds and certain species of cocci may be responsible for thickening of sweetened condensed milk.

Acid Products

In the spoilage of acid products a miscellaneous group of organisms is involved which includes non-sporing and sporing aciduric bacteria, yeasts and moulds. In most cases the organisms are readily controlled by relatively short processes at temperatures below 100 deg. C. (212 deg. F.).

Spore-forming Bacteria. Townsend (1939) has described spore-forming saccharolytic anaerobes of the *Clostridium pasteurianum* type which are responsible for gaseous spoilage in canned fruits and tomatoes. A strain studied by him grew under laboratory conditions in canned pear juice down to *p*H 3·55. Under commercial conditions however, the development of these organisms in canned products has not been observed below *p*H 3·7 (*cf.* Cameron and Esty, 1940). In phosphate buffer at *p*H 7·0, Townsend found that these organisms were destroyed in 40 minutes at 100 deg. C. (212 deg. F.). In tomato juice at *p*H 4·5 the destruction time was 20 minutes and in canned apricot juice at *p*H 4·15 it was 7 minutes. Speigelburg (1940) has also reported spoilage in canned pineapple by saccharolytic anaerobes resembling *Clostridium pasterurianum* which, however, were not as heat-resistant or as acid-tolerant as Townsend's strains.

Another aciduric, spore-forming organism which has been noted in America as a spoilage agent for acid products is *Bacillus thermoacidurens*. This organism, which was isolated by Berry (1933), is a thermophile with the general

characteristics of the " flat sour " type but is tolerant of an acid environment ; it has been responsible for spoilage in canned tomato juice. Spoilage of acid products due to spore-forming bacteria does not appear to have been observed in this country.

Non-sporing Bacteria. The most significant group of organisms of this type in the spoilage of acid packs are the Gram-positive, lactic acid-producing bacteria. The group includes both coccus and rod forms, some of which are gas producing. These organisms, which usually develop best under conditions of reduced oxygen tension, are widely distributed and are characteristically associated with the fermentation of vegetable material. Some members of the group are important in several industrial processes, being involved in the desirable fermentations which take place in cheese ripening and in vegetable pickling.

The gas-forming *Lactobacillus lycopersici*, which Bergey (1939) states is probably synonymous with *Lactobacillus brevis* (Orla Jensen), is well known as the cause of a vigorous fermentation in tomato ketchup, Worcester sauce, and similar products. Pederson (1929) described a number of organisms of this type which are associated with the spoilage of tomato products. Savage and Hunwicke (1923) reported a coccus, *Leuconostoc pleofructi*, which also belongs to the lactic group, as a spoilage agent in fruit juices. This organism, which is characterised by the production of slime in sugar solutions, was also noted by Pederson as a cause of spoilage in tomato products. The lactic acid group of organisms are controllable by moderate heat treatment at temperatures below 100 deg. C. (212 deg. F.).

Yeasts. Due to their low heat-resistance, yeasts are rarely involved in the spoilage of canned foods except in cases of gross under-processing or leakage. They are chiefly noted as causes of fermentation in acid sauces, jams and similar products.

Moulds. The noteworthy exception to the generalisation

that moulds are insignificant as spoilage agents in canned food is the species *Byssochlamys fulva*. This mould, described by Oliver and Smith (1933), is an important factor in the spoilage of canned and bottled fruits. It causes complete disintegration of the fruit due to the breakdown of pectinous material; in addition, infected cans sometimes swell through the production of carbon dioxide. Its optimum temperature for growth lies between 30 and 37 deg. C. (86–98·6 deg. F.). In relation to other mould species it is unusually heat resistant. Oliver and Smith (1933) state that it survives heating at 88 deg. C. (190·4 deg. F.) for 30 minutes. In processing experiments carried out by Hirst and McMaster (1932–1933), the spores of this mould survived when Victoria plums in A.2 cans were heated at 100 deg. C. (212 deg. F.) for 16 minutes. In other fruits the spores failed to survive these conditions. The degree of softening of the fruit caused by this species is unrelated to the amount of visible mycelium, complete disintegration taking place when to the naked eye, mycelium is not apparent. The species can tolerate a low oxygen tension, growth occurring in a vacuum of 20 in. According to Oliver and Rendle (1934), *Byssochlamys fulva* is present on fruit in the field and is probably derived from the soil. This mould appears not to have been observed in America where a notable heat-resistant, facultatively anaerobic *Penicillium* species has been isolated from canned blueberries by Williams, Cameron and Williams (1941). In blueberry juice, the thermal death-time of the sclerotia of this mould was 300 minutes at 85 deg. C. (185 deg. F.) although its ascospores were not unusually resistant, being destroyed in 15 minutes at 81 deg. C. (177·8 deg. F.). Growth occurred in a vacuum of more than 25 in. The source of this mould was found to be soil.

Commercial Sterility

From what has been said, particularly in relation to thermophilic bacteria, it will be realised that canned foods

are not invariably sterile. For low or medium-acid foods preserved by heat, the processes in general use are normally adequate to ensure the destruction of all bacteria but the most heat-resistant thermophiles and possibly some species of aerobic spore-formers. It is organisms belonging to these groups which are most commonly encountered in canned foods which, although unsterile, are considered to be commercially sound. The spores which survive the heat treatment are often incapable of development under normal storage conditions and this fact has led to the adoption of the term " commercial sterility " for canned foods.

Since the early days of the application of microbiology to food canning, the bacteriological condition of apparently sound canned foods has attracted the attention of a number of investigators. Tanner (1932) has reviewed some of the earlier reports and in connection with those where organisms of low heat-resistance were isolated, he has pointed out that sufficient attention may not have been paid to the mechanical condition of the containers. He also remarks that many of these earlier statements have been rendered invalid by the very marked improvements in canning technology which have taken place since the investigations were made.

It would seem, however, that there is still room for some improvement in the bacteriological condition of canned foods. In this country, the temperate storage conditions have tended to induce a certain degree of tolerance toward thermophilic contamination of many canned products. It seems not unlikely that some packers, not having experienced spoilage from this cause, may be unaware that their products are so contaminated. Under normal conditions, the rapid turn-over of stocks has rendered thermophilic contamination relatively unimportant. At the present time, however, when large stocks are being held for long periods and also exported to hot countries, such contamination assumes a greater importance than it has hitherto had for products packed in this country. If serious losses are to be avoided,

it is believed that greater care must be exercised in the selection of bacteriologically clean raw materials and it may even be necessary to sacrifice some degree of quality in the finished pack by increasing process times or temperatures in order to attain complete sterility.

REFERENCES

BARTON, L. H. G. 1938. *Food Manuf.*, **13**, 23.

BAUMGARTNER, J. G., and WALLACE, M. D. 1936. *Food Manuf.*, **11**, 10.

BERGEY, D. H. 1939. "Manual of Determinative Bacteriology." Baillière, Tindall and Cox, London.

BERRY, R. N. 1933. *J. Bact.*, **25**, 72.

BIGELOW, W. D., and ESTY, J. R. 1920. *J. Infect. Dis.*, **27**, 602.

BIGELOW, W. D., and CAMERON, E. J. 1932. *Ind. Eng. Chem.*, **24**, 655.

CAMERON, E. J., and ESTY, J. R. 1940. *Food. Res.*, **5**, 549.

CROSSLEY, E. L. 1938. *J. Hyg.*, **38**, 205.

CROSSLEY, E. L. 1941. *J. Soc. Chem. Ind.*, **60**, 131.

DONK, P. J. 1920. *J. Bact.*, **5**, 573.

FELLERS, C. R. 1927. *Univ. Wash. Publ. Fisheries*, **1**, 229.

HIRST, F. and MCMASTER, N. B. 1932–33. Ann. Rept. Fruit and Veg. Perserv. Res. Sta., Campden., p. 53.

JENSEN, L. B., WOOD, I. H., and JANSEN, C. E. 1934. *Ind. Eng. Chem.*, **26**, 1118.

JENSEN, L. B., and HESS, W. R. 1941. *Food Res.*, **6**, 75.

MCCLUNG, L. S. 1935. *J. Bact.*, **29**, 189.

MCMASTER, N. B. 1932–33. Ann. Rept. Fruit and Veg. Preserv. Res. Sta., Campden., p. 71.

MICHAEL, V. M., and TANNER, F. W. 1936. *Food Res.*, **1**, 99.

NICHOLS, A. A. 1936. *J. Soc. Chem. Ind.*, **55**, 78T.

NICHOLS, A. A. 1940. *J. Dairy. Res.*, **11**, 274.

OLIVER, M., and SMITH, G. 1933. *J. Botany*, **71**, 196.

OLIVER, M., and RENDLE, T. 1934. *J. Soc. Chem. Med. Ind.*, **53**, 166T.

PEDERSON, C. S. 1939. *N.Y. Agric. Expt. Sta. Tech. Bull.*, 150.

SAVAGE, W. G., and HUNWICKE, R. F. 1923 Food Invest. Bd. Dept. Sci. Ind. Res., London. Spec. Rept. No. 16.

SPEIGELBURG, C. H. 1940. *Food Res.*, **5**, 115.

TANNER, F. W. 1932. "Microbiology of Foods." Twin City Printing Co., Illinois.

TOWNSEND, C. T. 1939. *Food Res.*, **4**, 231.

TOWNSEND, C. T., ESTY, J. R., and BASELT, F. C. 1938. *Food Res.*, **3**, 323.

WERKMAN, C. H., and WEAVER, H. J. 1927. *Iowa State Coll. J. Sci.*, **2**, 57.

WILLIAMS, MERRILL, and CAMERSON, E. J. 1937. *Food Res.*, **2**, 369.

WILLIAMS, C. C., CAMERON, E. J., and WILLIAMS, O. B. 1941. *Food Res.*, **6**, 69.

CHAPTER V

THE PRINCIPLES OF HEAT-PROCESSING

THE heat-processing of canned foods is designed to ensure the destruction of all living micro-organisms capable of causing the food to become spoiled or unwholesome under ordinary conditions of storage. Coincidental with this achievement is the necessity for ensuring that the food has a normal palatability and the need for scientific adjustment of heat-processes arises because a process which is adequate from the cooking point of view may not be sufficient to ensure keeping quality. There needs to be a fine adjustment of the heat-treatment so that the spoilage organisms are destroyed without detriment to palatability. In order to arrive at this adjustment, consideration of three main factors is necessary. These are :—

The heat-resistance of the contaminating micro-organisms.

The chemical and physical nature of the contents of the can.

The rate at which heat penetrates to the centre or the most dense part of the contents.

The Heat Destruction of Micro-organisms

A knowledge of the fundamentals involved and the various factors which influence the heat-destruction of micro-organisms is essential for a proper understanding of the problems which arise in the heat-processing of canned foods.

All temperatures above the maximum for growth are lethal for micro-organisms. The rate at which death occurs is a function of time and temperature which vary with each other, the higher the temperature to which the cells are exposed the more rapid is their destruction. The lethal conditions for an organism cannot therefore, be expressed by temperature alone, that is, as a thermal death-point but

only as thermal death-time where the temperature and the time of exposure are stated.

The vegetative cells of bacteria, yeasts and moulds are destroyed almost instantly at 212 deg. F. (steam) and do not normally constitute a problem in the heat-processing of canned foods but the spores of certain species of bacteria are extremely resistant to heat and prolonged exposures to high temperatures are necessary for their destruction.

Cause of Death. The mechanism by which heat causes death of bacteria is still in doubt. It has been suggested that death may be due to the destruction of certain enzymes or to the coagulation of the cell proteins. Virtanen (1934) deduced that death is generally due to the destruction of bacterial enzymes. A more widely held view is that death results from the coagulation of the cell proteins and certainly there is abundant evidence that factors which affect protein coagulation also have a marked influence on bacterial heat-resistance. For example, an acid or alkaline reaction increases the coagulation of proteins and also causes a decrease in the heat-resistance of bacteria. In both processes water has an equally significant effect. The heat coagulation of albumen for example, varies with its water content, the most rapid coagulation occurring when the water content is high, becoming slower with decreasing water content. Where bacteria are concerned, the fact that they are more readily destroyed by moist heat than by dry heat is well known. Friedman and Henry (1938) have shown that the heat-resistance of the spores of different species of bacteria varies with their free water content. The markedly increased resistance of spores over the vegetative cells has been ascribed to the lower moisture content of the spore cell but Friedman and Henry produced evidence that the difference lies in the amount of water which is " bound " in the cell. They noted that a large part of the water in the spore cells was in the " bound " state and therefore not available to take part in the coagulation of the cell proteins. In an earlier

investigation (1937) they were unable to find any significant differences in the total water contents of spores and vegetative cells.

Rate of Destruction. In any given suspension, the cells do not show a uniform susceptibility to the effects of heat. Due to variation in the resistance of individual cells, the rate at which death occurs follows a logarithmic course—equal percentages of the surviving cells dying in each successive unit of time. If a suspension of bacteria or spores are heated and the number of viable cells is determined at intervals, the survivor curve generally approximates a straight line when plotted on semi-log paper. It is frequently found, however, that a few very resistant cells in a suspension do not conform to the general course of destruction and remain viable long after the majority of cells have died. For this reason, it has been advocated that where data is required for comparative purposes, for example in estimating the effect of a particular factor on the rate of heat destruction, the thermal death-time of 99·9 per cent. of the cells rather than the destruction time of all the cells in a suspension should be determined. The term " majority thermal death-time," is applied to the determination of the death-time of 99·9 per cent. of the cells; the " absolute thermal death-time " refers to the destruction of every cell.

Influence of Numbers. From the logarithmic course of death, it follows that the higher the initial number of cells the longer the time necessary to effect their complete destruction. It should be noted, however, that this holds only for cells from identical suspensions. Lang (1935) has stated that the heat-resistance of an individual suspension is a definite property which is rarely comparable with other suspensions, even when produced under the same conditions. There is, however, no doubt that for the same suspension the thermal death-time increases with increasing concentration of cells. In view of this relation of heat-resistance to the number of cells it is to be expected that the greater the

initial contamination in a given food the longer will be the period of heating required to sterilise it. Commercial experience has amply confirmed this view. As an example, Cameron and Yesair's (1931) work with sugar contaminated with thermophilic " flat sour " spores may be quoted. The results of their experiments clearly demonstrated that spoilage was related to the degree of contamination as shown in the following table :—

SPOILAGE IN CANS OF CORN (NO. 2) DUE TO THE PRESENCE OF " FLAT-SOUR " BACTERIA IN SUGAR

Processed at 250° F.	Percentage of Spoilage in Corn with :—		
	No Sugar	Sugar A1	Sugar A2
70 minutes . .	0	0	95·8
80 ,, . .	0	0	75·0
90 ,, . .	0	0	54·2

Sugar A1 contained approximately 60 " flat-sour " spores per 10 grams.

Sugar A2 contained approximately 2,500 " flat-sour " spores per 10 gm.

In the heat-processing of canned foods, the importance of reducing contamination to a minimum cannot be overemphasised. Any marked increase in the degree of contamination of raw materials or plant may result in a process which has been adequate for years suddenly becoming inefficient.

Other Factors Affecting Heat-Resistance. *Effect of Environment.* The environment in which the spores or bacteria are produced may have a considerable effect on their heat-resistance. Williams (1929) and others have reported that changes in the nutritive medium may produce deviations from the normal resistance. Increase in the incubation temperature is another factor which has been

stated to cause increased heat-resistance. In connection with the influence of environment it has been suggested that spores as they occur naturally, for example, in soil, may exhibit a different resistance to heat from those artificially produced. Hastings (1923) suggested that natural spores were the most resistant and Curran (1935) found that spores produced and aged in soil and oats were more resistant than those developed on artificial medium. Sobernheim and Mündel (1938) observed that soil with its natural spore content required 6 to 8 times longer heating for sterilisation than did a similar number of spores cultured from the same soil. Spores obtained from soil by extraction and remixed with heat-sterilised soil were also less resistant. They concluded that the higher resistance of spores in natural soil was due to some physico-chemical effect of the soil and not to any difference between soil and culture spores. In view of all the evidence available it would appear that laboratory findings on the heat-resistance of artificially produced spores should not be too readily interpreted as representing conditions existing in actual factory practice. Cameron, Esty and Williams (1936) carried out an investigation on a spore-forming organism which caused spoilage of canned beets. The process given the beets was 20 minutes at 116 deg. C. (240 deg. F.) which, from heat-penetration data, should have been sufficient to destroy spores with a resistance of approximately 10 minutes at that temperature. Under laboratory conditions, however, there was no survival after 5 minutes heating at 110 deg. C. (230 deg. F.). The investigators believed that the original resistance must have been materially higher than that indicated by the laboratory tests and the loss of resistance was presumed to be due to laboratory manipulation of the organism.

Effect of p*H.* Of the many factors which influence the heat-resistance of micro-organisms, the physical and chemical nature of the medium in which they are heated is of primary importance. The hydrogen ion concentration of the heating

medium may have a profound effect. For most spore-bearing bacteria maximum resistance generally occurs in the region of neutrality. Esty and Meyer (1922) found that *Clostridium botulinum* showed maximum resistance between *p*H 6·3 and 6·9. Williams (1929) observed that *Bacillus subtilis* spores were most resistant between *p*H 6·8 and 7·6. Aref and Cruess (1934) found that yeasts were slightly more resistant at *p*H 3·8–4·0. With one exception, however, the strains of yeast studied by Beamer and Tanner (1939) showed greater resistance at *p*H 6·8 than at *p*H 3·8. Gillespy (1936–1937) found that the maximum heat-resistance of the ascospores of *Byssochlamys fulva* occurred at about *p*H 5·0 the resistance at *p*H 3·0 was considerably greater than at *p*H 7·0. Baumgartner and Knock's (1940) experiments showed that non-sporing bacteria gave maximum heat-resistance at a *p*H value at which the resistance to disinfection by alcohol and stability in suspension were also at a maximum. It was concluded that at this *p*H value the hydration of the bacterial protein was at a maximum. Increase in the hydrogen ion concentration usually causes corresponding decrease in heat-resistance. Where canned foods are concerned, the effect of moderate changes in *p*H on the heat-resistance of spores appears to be variable. Esty and Meyer (1922) stated that above *p*H 5·0 some factor other than hydrogen ion concentration was more important in its effect on the time required to sterilise foods. With canned marine products, Lang (1935) found no correlation between *p*H and the heat-resistance of *Clostridium botulinum* spores within the range of 5·2–6·8. At *p*H 4·9, however, a marked reduction in resistance occurred.

Effect of Salt. Heat-resistance is affected by the presence of salt. Concentrations up to 4 per cent. were found by Viljoen (1926) to afford protection for the spores of several organisms heated in canned pea liquor. Esty and Meyer (1922) noted that 1–2 per cent. salt increased the heat-resistance of *Clostridium botulinum* spores. Above 8 per

cent. the resistance decreased. *Clostridium welchii* gave greater resistance in 3 per cent. salt when tested by Headlee (1931); at 10 per cent. concentration a definite decrease was noted.

Effect of Sugar, Proteins, etc. Sugar, glycerol and other non-toxic substances have been observed by several workers to protect micro-organisms from the harmful effects of heat. When such materials are present in sufficient concentration the protection is in some cases sufficiently marked to be significant in canning practice. Peterson, Levine and Buchanan (1927) found that yeasts were more readily destroyed at 100 deg. C. (212 deg. F.) in distilled water than in syrup. In syrup of 24° Be the thermal death-time was 6 minutes and in a syrup of 36° Be it was 28 minutes. Baumgartner and Wallace (1934) found that the survival time of *Escherichia coli at* 70 deg. C. (158 deg. F.) was increased from 4 to 6 minutes by 10 per cent. sucrose; in 30 per cent. sucrose the organism survived for 30 minutes. Fay (1934) and others observed a similar protective action by sugars. Protein materials such as gelatin and serum solids have likewise been reported as protective (Anzulovic 1932).

Organisms suspended in fat or oil are more difficult to destroy by heat than when suspended in aqueous fluids, probably because the conditions resemble dry heat sterilisation. Spores protected in this manner may survive heat-treatment which would normally be adequate for their destruction in an aqueous medium and eventually germinate if they come in contact with a nutrient medium. Lang (1935) observed that *Clostridium botulinum* spores survived heating beyond all reasonable expectation when heated in oil suspensions and pointed out that the eventual germination of oil-trapped spores constitutes a definite problem in sterilisation.

Effect of Lethal and Inhibitory Agents. The presence in the heating medium of any lethal or inhibitory substance can be expected to cause a reduction in heat-resistance. Berry,

Jensen and Siller (1938) and Coulthard (1939) have shown the value of small amounts of germicides in increasing the efficiency of low-temperature sterilisation. Pederson and Beavens (1940) state that 22 parts per million of sulphur dioxide may lower the pasteurisation temperature of fruit juices by 10 deg. F. According to Gillespy (1940), the lethal effect of heat on the ascospores of *Byssochlamys fulva* is enhanced by as little as 2 to 10 parts per million of sulphur dioxide at *p*H values below 3·7. It seems not improbable that the essential oils of certain spices such as cloves and mustard which are known to be toxic to micro-organisms at normal growth temperatures may, at processing temperatures and in low concentrations, have an appreciable effect in reducing heat-resistance.

In view of the various influences which may exist in the heating medium it follows that the resistance in food may be quite different from that in buffer solutions. Arising out of this difference is the necessity, where food-processing problems are concerned, for heating the test organisms in the actual food. The ratio of the resistance in food to the resistance in phosphate buffer is known as the food-phosphate factor and this is sometimes applied for the purpose of comparing the resistance of suspensions.

Dormancy. The delayed germination or dormancy of heated spores has been observed and studied by many workers and is a problem of particular significance in the microbiology of canned foods. Spores which have received sub-lethal exposures to heat sometimes remain dormant in favourable media for long periods. Dickson (1938) has recorded the delayed germination of heated *Clostridium botulinum* spores of more than 6 years. Esty and Meyer (1922) repeated that heated spore suspensions (*Clostridium botulinum*) remained dormant for 378 days at 36–37 deg. C. (96·8–98·6 deg. F.) These represent extreme cases but dormancy periods of weeks are a common occurrence. The cause of dormancy is still a matter for conjecture. It has

been stated to be due to the selective action of heat on the cells. Those possessing thick, relatively impermeable membranes survive and show delayed germination. Topley and Wilson (1936) believe that a more probable theory is that as the result of heat-treatment the cells suffer some injury which interferes with their normal reproduction.

Estimation of Thermal Death-Times. In view of the many factors which influence the heat-resistance of micro-organisms it is evident that a very carefully standardised technique is necessary for securing heat-resistance data. Many methods have been proposed but those devised by Bigelow and Esty (1920) and Esty and Williams (1924) are chiefly employed. Bigelow and Esty's method, known as the single tube method, involves heating a definite amount of a suspension of the organism or its spores in small glass tubes (7 mm. bore, wall 1 mm. thick). After sealing in a flame, the tubes are immersed in an oil bath of constant temperature and at short intervals a single tube is removed, rapidly cooled and cultured into a suitable medium. Later, Esty and Williams proposed the multiple tube technique in which, instead of a single tube being removed from the bath, at short intervals, a large number of tubes (25–30) are removed at each of four widely spaced times. The tubes are cooled and cultured into suitable medium. For each heating period the percentage of tubes giving growth is plotted on semilog paper and from the resulting straight line the thermal death-time of the organism at the temperature used can be found by extrapolation. The method has the effect of reducing the incidence of " skips " which occur when Bigelow and Esty's technique is used, " skips " being the term given to tubes which give no growth on sub-culture although heated for a shorter time than tubes containing viable organism. Esty and William's method is the one most frequently employed in making process calculations. For full details of the technique the original paper should be consulted.

Heat Penetration

In the heat-processing of canned foods it is always assumed that the heat-resistant micro-organisms will be located at the centre or the slowest-heating part of the can contents and the calculation of process times is partly based on the rate at which heat is transferred to this point.

The rate of heat-penetration can be determined by means of the thermo-electric couple. This instrument depends on

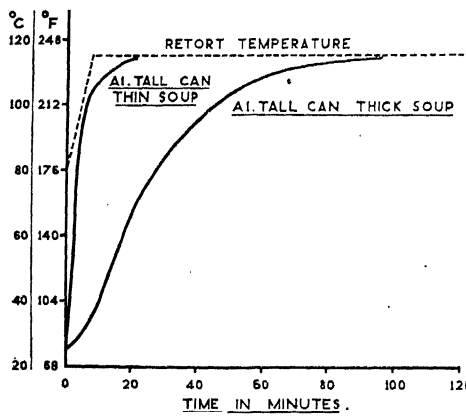


FIG. 5. Heating curves of thin and thick soups.

the principle that when two dissimilar metal wires such as copper and constantan are joined at both ends to form a circuit and one junction is at a higher temperature than the other, a current is set up, the magnitude of which depends on the temperature difference between the two junctions.

In practice, one junction (the hot junction) is inserted in the centre of the can or in the slowest-heating part of the contents and the second (the cold junction) is held at a steady temperature. A microammeter is put into circuit and as the temperature of the can in the retort is raised, the change in current is indicated on the microammeter. It is necessary that the apparatus be previously standardised

by exposing both junctions to known temperatures, for example 0 deg. C. (32 deg. F.) and 100 deg. C. (212 deg. F.). Since the e.m.f.-temperature difference relation is more or less linear, any reading on the microammeter can then be related to temperature. In the actual determination of heat-penetration rates, the time-temperature curve is obtained by taking the current reading at regular intervals. Theremo-electric couples designed for determining heat-penetration rates in canned foods are marketed by several instrument makers.

The rate of heat transference in canned food is controlled by the physical character of the food, the difference between the retort temperature and that of the food and the size and shape of the container.

For any canned food, the rate of heat-penetration is affected by the temperature gradient between the can and the retort, the rate becoming slower as the temperature difference decreases. From this it follows that increase in the retort temperature results in more rapid heat-penetration with consequent decrease in sterilising time. Due to the effect of temperature gradient on the rate of heat-penetration, a cold-filled can will reach retort temperature in about the same time as a partially heated can, but this in no way detracts from the value of hot-filling when processing canned foods since hot cans will reach a lethal temperature more rapidly than those cold-filled.

Two mechanisms are involved in the heating of canned foods. In liquids, heat transference takes place mainly by convection and the rise in temperature is rapid since there is constant movement of the material so that at any time during the heating temperature differences within the can are small. Any substance which retards convection currents decreases heat transference. Thus, the presence of sugar in high concentration causes a decrease in heat-penetration by increasing the viscosity of the liquid.

Agitation of the cans during heating markedly increases

the rate of heat-penetration in liquid packs by transferring the heated portion of the contents to the cooler regions of the can. For products such as cream, which is adversely affected by heat, agitation is applied during processing, the cans being loaded into cages which are rotated in a retort or a continuous, agitating cooker is employed.

Solid foods heat by conduction and the process is relatively slow since there is no transfer of material from the hot to the cooler part of can ; the rate at which the heat is conducted decreases as the temperature difference between the centre of the can and the retort becomes smaller. In some products both heating mechanisms may operate, as for example, materials containing starch where in the early stages of heating convection is the main factor involved in heat transfer but with the transition of the product to a gel form, heating continues mainly by conduction. The rate of heat-penetration decreases as the starch concentration increases up to 6 per cent., but concentrations above this figure cause no further decrease (Bigelow and others, 1920).

With solid materials packed in liquids the ratio of solid to liquid and also the arrangement of the solids in the liquid will affect heat-penetration ; the presence of channels which permit convection currents will obviously facilitate the transfer of heat and solids loosely packed in a liquid will heat more rapidly than if tightly packed.

The shape and size of the container for any given product is very important since the rate of heat-penetration is affected by the ratio of the surface area of the container to its volume. Small cans heat more rapidly because the surface area exposed to heat is large in relation to the volume of the can.

In connection with the rate of heat transference in foods attention is directed to the " high-short " methods of processing. The subject of " high-short " processing, which as the term implies, involves the use of high temperatures for short periods, has been reviewed by Ball (1938) and

while the commercial application of the principle is at present limited to such products as fruit juices, Ball states that—"the indication is that these methods will in time supplant present ones." The principle on which "high-short" is based is that in proportion to their lethal effects on spoilage micro-organisms, high processing temperatures have less effect on quality of foods than lower temperatures. Since "high-short" processing implies very rapid heat-penetration, it is applicable only to those products heating rapidly by convection or by agitation.

Standards of Processing

Experience has shown that the micro-organisms capable of causing spoilage in canned foods with *p*H values below 4·5 are of low heat-resistance and are readily controllable by temperatures below 100 deg. C. (212 deg. F.). Consequently, for products in this class, pressure processing is not applied. Above *p*H 4·5 heat-resistant spore-forming bacteria become significant as spoilage agents; furthermore, the growth of *Clostridium botulinum* may occur. To ensure the destruction of heat-resistant spore-forming organisms in foods with higher *p*H values than 4·5, pressure processing at temperatures above 100 deg. C. (212 deg. F.) is required. Although it seems reasonable to anticipate that in food products with *p*H values in the range 4·5–5·0 the heat resistance of spore-forming bacteria would be reduced, at the present time there appears to be little or no data available in this connection.

In formulating processes for medium-acid and low-acid foods the greatest difficulty lies in the high heat-resistance of the thermophilic organisms. For most foods, processing which is adequate to ensure the destruction of these organisms results in deterioration of quality through overcooking. The ideal of complete sterility is, therefore, often unattainable. In such cases the canner's main concern is the production of foods which are safe from the public health point of view.

For medium-acid and low-acid foods the achievement of this aim requires the destruction of the most heat-resistant pathogen—*Clostridium botulinum*—and this has gained general acceptance as the minimum standard of processing for all products in these groups. As a standard on which to base process times the maximum heat-resistances observed for the spores of *Clostridium botulinum* by Esty and Meyer (1922) are generally accepted. In their experiments, the highest resistances for spores produced under the most favourable conditions and heated in phosphate buffer solution at *p*H 7·0 were as follows:

100° C. (212° F.)	330 minutes
105° C. (221° F.)	100 ,,
110° C. (230° F.)	32 ,,
115° C. (239° F.)	10 ,,
120° C. (248° F.)	4 ,,

Any process for foods in the low-acid or medium-acid group should be sufficient to destroy spores with a thermal death-time equal to those given by Esty and Meyer. Their results, however, were obtained with spores suspended in buffer in small tubes in which the time taken to reach the desired temperature is negligible. In processing canned foods the problem is to evaluate the lethal effect of the period during which the temperature of the can contents is rising to its maximum; especially where the heat-penetration rate is slow the temperature continues to rise during most of the process time. Since, for any organism, all temperatures above the maximum for growth may be considered as being lethal, the sterilising effect of a process commences as soon as the food reaches a lethal temperature. It is in this connection that the temperature of the cans at the time of entering the retort becomes so important, since hot cans will reach a lethal temperature more rapidly than cool cans. For practical purposes a lethal effect on spore-forming organisms in low-acid and medium-acid foods is

usually assumed to commence at 93·3 deg. C. (200 deg. F.). In assessing the total lethal effect of a process therefore, the period during which the temperature of the food is rising from 200 deg. F. to the maximum reached in the retort and also the period while it is cooling to 200 deg. F. must be taken into consideration.

Formulation of Processes

Two main procedures are used for estimating the lethal effect of a process. In the first, the canned food is inoculated with the spores of an organism representative of the most heat-resistant type likely to occur as a contaminant. The inoculated cans are then subjected to various processes after which the viability of the organisms is determined by culture and incubation tests. From the results obtained the time and temperature conditions which will destroy the organism are deduced.

In the second method, the value of the process is computed mathematically, basing the calculation on the thermal death-time of the organism at various temperatures and the rate of heat-penetration into the can. Methods in which these data are used for calculating and assessing processes have been devised by Bigelow, Bohart, Richardson and Ball (1920) and Ball (1923, 1928). It should be remarked that the conclusions from these mathematical calculations are customarily checked by experimental inoculation of cans with spores of known heat-resistance. The inoculated cans are then processed and tested by incubation and cultures.

Bigelow's method (known as the "general" method) is founded on the fact that each point on the heat penetration-cooling curve for a can of food represents a lethal rate value for the organism studied and involves the construction of a lethality curve. First, the thermal death-times of the test organism are determined for several temperatures within the processing range. From the data obtained a curve is constructed on semi-log paper, using the log scale for time

and the linear scale for temperature. From this curve can be found the thermal death-time for the organism at any temperature within the processing range. The lethal rate for any given temperature is the reciprocal of the number of minutes required to kill the organism at that temperature. If the rate of heat-penetration and cooling is known, any temperature reached during the process may be given a lethal rate value ; if a curve is constructed as for a heating-cooling curve in which lethal rates are substituted for temperatures, a lethality curve is obtained. When the area beneath the lethality curve is equal to unity the process is considered to be adequate with respect to the organism studied. This method of calculating process values has been criticised on the grounds that it is laborious and can be applied only when the conditions relating to can size, retort temperature and initial temperature of the can to be processed are identical with those under which the heating-cooling curve data were secured. Schultz and Olsen (1940) have suggested the use of special co-ordinate paper with which less effort is entailed in making the calculations. They also give formulæ for converting heat-penetration data obtained under one set of initial and retort temperatures to data corresponding to different initial and retort temperatures.

Ball developed a formula method for calculating processes by which any given thermal death-time and heat-penetration data can be applied to any can size or retort temperature provided the thermal death-times and the rate of heat-penetration approximate straight lines when plotted on semi-log paper. These curves are defined mathematically in Ball's method.

This method is widely used in the study of processing problems in America but it has received some criticism on the ground that the calculations are very involved and require considerable mathematical knowledge, although nomograms constructed by Olsen and Stevens (1939) have simplified its application by permitting the calculations to

be carried out graphically. Another objection is that it is not applicable to all products. Taggart and Farrow (1941) state: "The condition that the curves should be straight lines when plotted on semi-logarithmic paper is satisfied by the heating curve when the heat transmission within the pack is solely by conduction. . . ." In their paper, Taggart and Farrow give a simple procedure based on the "general" method, for estimating the lethal effect of a process with respect to *Clostridium botulinum*. Their method involves the construction of a lethality curve for this organism on special graph paper. A lethal rate table based on Esty and Meyer's thermal death-times for *Clostridium botulinum* spores in buffer at *p*H 7·0 is given by Taggart and Farrow:—

LETHAL TIMES AND LETHAL RATES OF *Clostridium botulinum* (INTERPOLATED)

mperature ° F.	Lethal Time	Lethal rate $r = \frac{1}{\text{lethal time}}$
200	1514	0·00066
205	794	0·00126
210	417	0·00240
215	219	0·00457
220	115	0·00870
225	60	0·0167
230	32	0·0312
231	28·8	0·0347
232	25·1	0·0398
233	21·9	0·0457
234	19·3	0·0518
235	17·0	0·0588
236	14·9	0·0671
237	13·0	0·0769
238	11·5	0·0870
239	10·0	0·100
240	8·7	0·115
245	4·6	0·217
250	2·4	0·417

Where the area under the lethality curve for any process exceeds unity and the curve is constructed for the lethal rates shown above, that process is adequate to destroy the spores of *Clostridium botulinum* or any other organism with

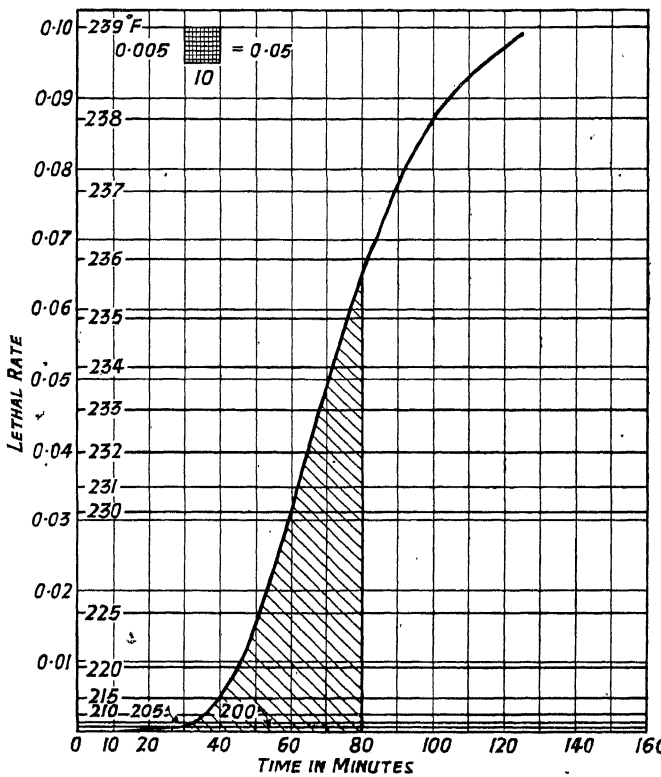


FIG. 6.

thermal death-times in the food equivalent to those reported by Esty and Meyer for spores in buffer at *p*H 7·0. The area under the curve is readily determined by counting the number of squares. With the use of Taggart and Farrow's simplified graph-paper it is unnecessary to refer to the lethal

rate table. The temperature lines on the vertical scale are drawn at distances from the horizontal time scale which are proportional to the lethal rates at the corresponding temperatures and it is only necessary to plot the heat-penetration data on the paper, the area under the curve giving the lethal effect of the process. This method ignores the cooling period which is regarded as an additional safety factor. Fig. 6 shows a typical lethality curve reproduced from Taggart and Farrow's paper.

REFERENCES

ANZULOVIC, J. V. 1932. *J. Bact.*, **23,** 56.

AREF, H., and CRUESS, W. V. 1934. *J. Bact.*, **27,** 443.

BALL, C. O. 1923. *Bull. Nat. Res. Council* 7, Part 1, No. 37.

BALL, C. O. 1928. *Univ. Calif. Pub. in Publ Health,* **1,** 15.

BALL, C. O. 1938. *Food Res.*, **3,** 13.

BAUMGARTNER, J. G., and KNOCK, G. G. 1940. *J. Soc. Chem. Ind.*, **59,** 53T.

BAUMGARTNER, J. G., and WALLACE, M. D. 1934. *J. Soc. Chem. Ind.*, **53,** 294T.

BEAMER, P. R., and TANNER, F. W. 1939. *Zentralbl. Bakt.*, 11 Abt., **100** (9/13); 202.

BERRY, H., JENSEN, E., and SILLER, F. K. 1938. *Quart. J. Pharm.*, **11,** 729.

BIGELOW, W. D., BOHART, G. S., RICHARDSON, A. C., and BALL, C. O. 1920. *Nat. Canners' Ass. Bull.*, 16L.

CAMERON, E. J., and YESAIR, J. 1931. *Canning Age,* **12,** 239.

CAMERON, E. J., ESTY, J. R., and WILLIAMS, C. C. 1936. *Food Res.*, **1,** 73.

COULTHARD, C. E. 1939. *Pharm. J.*, **142,** 79.

CURRAN, H. R. 1935. *J. Infect. Dis.*, **56,** 196.

DICKSON, E. C. 1928. *Proc. Soc. Exp. Biol. and Med.*, **25,** 426.

ESTY, J. R., and MEYER, K. F. 1922. *J. Infect. Dis.*, **31,** 650.

FAY, A. C. 1934. *J. Agric. Res.*, **48,** 453.

FRIEDMAN, C. A., and HENRY, B. S. 1938. *J. Bact.*, **36,** 99.

GILLESPY, T. G. 1936–1937. Ann. Rept. Fruit and Veg. Preserv. Res. Sta., Campden, 68.

GILLESPY, T. G. 1940. Ann. Rept. Fruit and Veg. Preserv. Res. Sta., Campden, 54.

HASTINGS, E. G. 1923. *J. Infect. Dis.*, **33,** 526.

HEADLEE, M. R. 1931. *J. Infect. Dis.*, **48,** 468.

HENRY, B. S., and FRIEDMAN, C. A. 1937. *J. Bact.*, **33,** 323.

JENSEN, L. B., and HESS, W. R. 1941. *Food Manuf.*, **16,** 157.

LANG, O. W. 1935. *Univ. Calif. Pub. in Publ. Health,* **2,** No. 1, 1–182.

OLSEN, F. C. W., and STEVENS, H. P. 1939. *Food. Res.*, **4,** 1.

PEDERSON, C. S., and BEAVENS, E. A. 1940. *Food Ind.*, **12**, 61.
PETERSON, E. E., LEVINE, M., and BUCHANAN, J. H. *Iowa State Coll. J. Sci.*, **2**, 31.
SCHULTZ, O. T., and OLSEN, F. C. W. 1940. *Food Res.*, **5**, 399.
SOBERNHEIM, G., and MÜNDEL, O. 1938. *Z. Hyg.*, **121**, 90.
TAGGART, R., and FARROW, F. D. 1942. *Food*, **11**, 13.
TOPLEY, W. W. C., and WILSON, G. S. 1936. "Principles of Bacteriology and Immunity." Arnold & Co., London.
VILJOEN, J. A. 1926. *J. Infect. Dis.*, **39**, 286.
VIRTANEN, A. T. 1934. *J. Bact.*, **28**, 447.
WILLIAMS, O. B. 1929. *J. Infect. Dis.*, **44**, 421.

CHAPTER VI

TYPES OF SPOILAGE

CANNED foods are classified as spoiled when, for any reason, the food has undergone a deleterious change or the condition of the container renders such change possible. Spoilage may arise from a variety of causes which include the activity of micro-organisms, chemical reaction between the contents and the container, faulty technique in canning practice, rough handling and poor storage conditions. Spoiled cans may present a perfectly normal external appearance or they may show obvious abnormalities such as varying degrees of distortion. In certain instances, for example, where distortion of the can has arisen as the result of faulty canning practice or retort manipulation, the contents may be quite wholesome. In spite of this such cans are considered to be spoiled since they are visually indistinguishable from cans which have spoiled as the result of microbial activity and are, therefore, unsaleable.

A can with its ends bulged by positive internal pressure due to gases formed as the result of microbial or chemical activity is termed a " swell " or " blower." A " hard swell " is one in which the ends of the can are permanently and firmly extended; if the ends can be moved by thumb pressure but cannot be forced back to the normal position the can is referred to as a " soft swell." The term " springer " is used to describe a can in which one end is bulged but can be forced back into the normal position whereupon the opposite end bulges. A " flipper " is a can of normal appearance in which an end " flips " out when the can is struck against a solid object; the end snaps back to the normal position when very light pressure is applied. It should be noted that all swelled cans pass successively through the " flipper " and " springer " stages.

The chief specific causes of spoilage may be tabulated as follows :—

Microbial :	Under-processing.
	Infection resulting from leakage through seams.
	Pre-process spoilage.
Chemical :	Hydrogen swell.
Physical :	Faulty technique in retort operation.
	Under-exhausting.
	Over-filling.
	Panelling.
Miscellaneous :	Rust.
	Damage.

Under-processing. In under-processed packs the activity of the surviving organisms may result in gas production which causes the can to become a " swell " or the contents may undergo " acidification " or some other undesirable change affecting quality without production of gas. When growth of micro-organisms occurs without gas production, the affected cans have a normal appearance externally and spoilage is only detectable after the can has been opened.

Unless the pack has been grossly under-processed, it will generally be found that the spoilage is due to a single type of organism. In low-acid or medium-acid products this is invariably a spore-forming species. In acid products which have not been subjected to a pressure process, yeasts or mould or aciduric non-sporing bacteria may be found ; non-aciduric spore-formers may also be present but these organisms have no significance since they are incapable of growth in acid environment. There are, of course, exceptions but as a general rule the presence of a single species in a can free from obvious mechanical defects is indicative of under-processing.

The first step in the bacteriological investigation of spoilage of this kind is the identification of the organism. In the

initial stages complete identification is not required, but it is at least essential to determine to what group the organism belongs in order that its origin may be traced without delay. Thermal death-time estimations carried out in the food involved and in buffer solution may enable adjustment to be made to the processing conditions if quality considerations permit. In attempts to trace the source of the organism and, if possible, to effect its elimination, all raw materials should be investigated. In addition to the main ingredients, materials such as colouring solutions and spices should not be overlooked. Spoilage in canned chopped ham due to spore-forming bacteria carried by spices has been reported by Jensen, Wood and Jansen (1934). Crossley (1938) found colouring materials and spices to be a prolific source of bacteria, including putrefactive anaerobes. In America, Yesair and Williams (1942), although finding heavy bacterial contamination in a wide range of spices and herbs found thermophilic aerobic spores to be relatively low in number and failed to detect putrefactive anaerobes.

Cameron (1938) has emphasised the importance of contamination from plant and equipment, especially where thermophilic " flat sour " type spores are concerned. He remarks that the chances of spoilage are greater in complicated plants in which there are heated blending tanks, mixers, reservoirs, etc., wherein thermophiles may develop. Improperly cleaned plant in which over-night development of heat-resistant organisms has occurred has also been noted as a factor in spoilage outbreaks.

Soil carried by vegetables may be an important source of spoilage organisms. It has been noted that infection with a particular organism may be confined to vegetables derived from a single locality. In the writer's experience, carrots supplied by a certain farm were found to be heavily infected with a gas producing thermophile which caused an outbreak of spoilage ; carrots from other localities, some not far removed from the infected farm, were free from this organism.

With reference to infection carried by vegetables, special blanching methods have proved useful. Preliminary treatment of vegetables by boiling in 1 per cent. citric acid solution for about 15 minutes has been found to be very effective in reducing contamination by heat-resistant thermophilic spoilage organisms.

Leakage through Seams. Among the causes of spoilage in canned foods, that arising from leakage of can seams is probably of greater economical importance than any other. The industry's annual wastage through leaker spoilage must be very considerable, but improvements in canning technique and the growth of technical supervision should enable the losses to be reduced.

The micro-organisms infecting canned foods as the result of post-process leakage of the containers may be of widely varying types including cocci, non-sporing rods and sporing rods. Yeasts are only rarely found and moulds are present only when the container is grossly defective. The main source of the organisms is the cooling water. Air-borne organisms are probably of little significance as a cause of leaker spoilage, air-cooled cans invariably showing a lower proportion of spoiled cans than those cooled in water unless the latter has been disinfected by chlorination or some other means.

In cans which have leaked, there is often present more than one type of organism. Leaking cans may, at times, be contaminated by a solitary species such as a sporulating organism. This may apply particularly where cans have been cooled in chlorinated water in which there will be a tendency for the bacterial content to be reduced to resistant spore-formers. In pressure-processed cans the presence of viable cocci or non-sporing rods is certain indication of post-process leakage. In this connection it is well to remember that microscopic examination of the contents may reveal cocci which, however, may have been present in the raw material at the time of canning and destroyed by the heating process. Bacteria are often microscopically demonstrable

in the contents of sound cans. Microscopic examination is invaluable in the examination of spoiled cans but should always be supplemented by cultures to determine whether the organisms observed are viable. Since the majority of the organisms present in cooling water and air have an optimum growth temperature of 20–25 deg. C. (68–77 deg. F.) cultures at this temperature should always be included in the examination of suspected leakers. Many of the organisms found in water and air grow poorly or not at all at 37 deg. C. (98·6 deg. F.) and it is quite possible that cultures prepared from infected cans and incubated at this temperature may fail to reveal the presence of viable organisms in some cases.

In solid products, such as meat rolls, the infecting organisms are more likely to be on the surface of the contents and cultures prepared with inoculum carefully taken from the centre may prove to be sterile. It is important, therefore, when examining this type of product for leaker spoilage, to ensure that the inoculum includes material from the surfaces.

In all leaker examinations, the use of solid media in addition to the liquid type is advisable since it facilitates better assessment of the degree to which the various organisms are present, and also obviates the possibility in liquid media of overgrowth of a slow-growing species by the rapidly growing types. Another advantage which accrues from the use of solid media is the elimination of doubt concerning the possibility of contamination of the cultures during their preparation. The finding of numerous colonies of cocci or non-sporing rods on solid media makes it almost certain that they originated from the sample.

The measures to be adopted for minimising the leaker type of spoilage consist of making the can seams as near the ideal as possible, maintaining the bacterial content of the can-cooling water at a low level, careful manipulation of the retorts during the cooling process, and ensuring proper exhausting of the can, thereby minimising seam strain.

Since the bulk of the cans which spoil through leakage become infected during the water-cooling process, the bacteriological quality of cannery cooling water becomes highly significant. During the water-cooling process which immediately follows heat-treatment, a vacuum is formed in the can as a result of the condensation of water vapour and contraction of the residual air and the contents. Although the can seams may be of normal quality they are subject to considerable stresses during retorting and these may cause the seam to leak sufficiently to permit the entrance of minute amounts of cooling water; the chances of bacteria being sucked into the can will obviously increase with the number of bacteria in the water. Where, for economical reasons, water is recirculated for use, the bacterial population may reach many millions per ml. Especially during warm weather is this likely to occur; there are usually ample bacterial nutrients in the water in the form of food washed from the outside of the cans. In America, Scott (1937) and others have demonstrated that as a result of reducing the bacterial contamination of cooling water by chlorination, spoilage of canned foods could be reduced to one-fifth or less of that which occurred among cans cooled in highly contaminated water and this agrees with general experience in the industry.

Chlorination may be carried out with chlorine gas or hypochlorite solution but the dosage must be carefully controlled. The standard for cooling water should be similar to that for a potable water (about 100 organisms per ml.) and if sufficient chlorine is added to give a residual amount of 1 part per million of water, it will generally be found that the bacterial contamination will be reduced to this level. In exceptional circumstances, as when the water contains much organic material, the chlorine dosage may need to be considerably increased above that for clean water in order to give a residue of 1 part per million.

Although chlorination of cooling water is a valuable

means of reducing spoilage resulting from leakage it cannot be regarded as a safeguard for poor seaming. Chlorination should be applied only when laboratory tests confirm that spoilage is due to leakage and that seams are of reasonable quality. The cooling of poorly-seamed cans in almost sterile water may actually be disadvantageous in that defective cans which would normally become infected by cooling water and probably be eliminated before leaving the factory are released for distribution and may eventually undergo spoilage by bacteria or corrosion.

Pre-process Spoilage. Spoilage of this type is an example of faulty canning practice. Particularly during warm weather, development of fast-growing organisms may occur if there is any undue delay between filling the container and heat-processing. This especially applies to cold-filled packs such as meat pastes. Processing may subsequently sterilise the pack but the liberation of gas produced by the organisms during the lag period before processing may cause swelling or flipping of the cans. A typical case of pre-process spoilage in canned meat has been described by McClung and Wheaton (1936). The spoilage organism was *Clostridium welchii* which survived the preliminary cooking of the meat. Rapid growth of the organism in the warm meat resulted in the production of sufficient gas to swell the cans. No viable organisms could be isolated from the cans after processing.

In spoilage of this type, cultures prepared from processed cans are invariably sterile, but microscopically, numerous organisms can be demonstrated. Similar bacteriological findings may obtain with cans which have swelled following under-processing or leakage and in which the bacteria have died out. The dying-out of organisms is termed autosterilisation. Cultures prepared from such cans are sterile but numerous organisms may be seen microscopically, although the cells occasionally disappear entirely. Thermophiles are particularly liable to undergo rapid autosterilisation. It is sometimes difficult to distinguish these

two conditions but it is usually possible to arrive at a correct diagnosis by noting the condition of the organisms in stained smears. Where organisms have undergone autosterilisation the cells show uneven staining and other degenerative changes such as gross alterations in morphology. Where death has resulted from heat processing, the cells usually stain crisply and do not appear degenerate.

Hydrogen Swells. This description is applied to cans which bulge as the result of the formation of hydrogen following internal corrosion of the can. Varying degrees of bulging may be observed ranging from the "flipping" to the "hard swell" stage and under certain conditions corrosion may result in actual perforation of the can.

Hydrogen swells, the contents of which may be quite harmless, are chiefly associated with foods containing organic acids, such as fruits, and their formation depends upon a number of factors including the efficiency of the exhaust, the size of the headspace in the can and storage temperature. Considerable losses due to hydrogen swelling have been experienced, especially with canned fruits and the corrosion of tinplate has been extensively investigated in America and in this country. No more than a brief outline of the subject can be given here but there are available numerous publications giving more detailed information.

Due to the normal imperfections which exist in the tin coating or to scratches or damage sustained in the can making process, small areas of iron are exposed and when the two metals are in contact with an electrolyte (*e.g.*, fruit juice) an electrocouple is formed. The corrosion of tinplate is therefore a more complicated process than the corrosion or tin or iron alone. The main factor which influences the course of corrosion of tinplate is the polarity of the metals in the couple. According to Hoar (1936), the chief factors which govern the polarity are the presence or absence of oxide film on the metal surfaces and the ability of the electrolyte to remove tin ions as complexes. After dissolution or

disintegration of the oxide film and provided the electrolyte contains such anions as citrate or oxalate with which tin can form stable complexes, tin becomes anodic, the attack being more or less confined to the tin surface while the iron base is protected. If stable complexes are not formed tin remains cathodic and the iron is attacked and may eventually be perforated. The importance of oxygen in the corrosion process lies in its action as a depolariser. Theoretically, hydrogen should be evolved as a gas when displaced from solution by a metal but such evolution is very slow at the tin surface unless oxygen or some other depolariser is present. If hydrogen is not evolved it exerts a back e.m.f. and thus opposes further solution of the iron. The rate of attack is therefore much slower in the absence of oxygen. Anthocyanin pigment in red fruits also acts as a depolariser and various other substances are known to accelerate or retard corrosion. Protein and tin ions act as inhibitors and sulphite as an accelerator. The rate of metal attack is also influenced by the composition of the base plate and the method of manufacture. It is generally agreed that cold-reduced plate is more resistant than that made by the hot-rolling process. Contrary to normal expectation, lacquered cans are more prone to perforation than plain cans due to the main fact that the areas of exposed iron are not afforded cathodic protection and also protection by dissolved tin. It is also observed that products which are only slightly acid are often more corrosive than others of higher acidity. In this connection it is known that at low acidity the tin coating is more readily attacked than at high acidity; another factor is the decreased activity of corrosion inhibitors at low acidity. For more detailed information concerning tinplate corrosion, publications by Morris and Bryan (1931), Morris (1933), Hoar (1936) and Hirst and Adam (1937) should be consulted.

In the examination of hydrogen swells, inspection of the interior of the cans may reveal little since the degree of

corrosion often appears as great in normal cans. Analysis of the headspace gases is the only reliable method, the proportion of hydrogen in hydrogen swells usually being at least half of the total volume of gas present.

It is possible that the losses from hydrogen swells may increase with the substitution of tinplate by blackplate (lacquered untinned steel). Once the lacquer film is broken

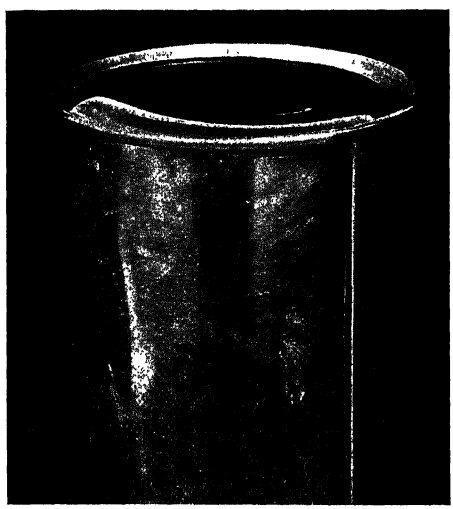


FIG. 7. Peaking.

the attack on the metal will in general be greater than with tinplate. Present indications are that the effect of oxygen in the headspace on the corrosion of blackplate may be even more marked than with tin plate. The purpose of using blackplate is, of course, to effect economies in tin consumption.

Incorrect Retort Operation. Too rapid decrease of steam pressure at the conclusion of processing results in setting up high pressure inside the cans and this may cause such severe straining and distortion that when cooled the cans have

the general appearance of "swells." It will be found that strained cans produced in this manner have no positive internal pressure and the ends can be forced back more or less to their normal position. A diagnostic feature of cans which have been subjected to abnormal strain in processing is the "peaking" or deformation of the panelling or expansion rings on the ends of the cans (see Fig. 7). Whilst moderately strained cans may not warrant classification as spoiled, every attempt should be made to eliminate them since such strain is highly conducive to leaker spoilage. The necessity for less rapid reduction in steam pressure or better still, pressure cooling, is indicated where straining occurs.

Under Exhausting. During heat processing, improperly exhausted cans suffer severe strain due to the excessive internal pressures set up by the expansion of the entrapped gases. The appearance of under-exhausted cans may vary from slight flipping to gross distortion, depending on the amounts of residual air and gases evolved from the contents, and the size of the headspace, but even in grossly distorted cans the internal pressure seldom exceeds a few pounds per square inch.

Usually, most of the excess gas in the headspace of under-exhausted cans is air, exceptions being found with vegetable and to a lesser extent with meat packs from which carbon dioxide is liberated and may be found in abnormal amounts. Normally, the bulk of the headspace gas is nitrogen. The proportion of oxygen found in canned foods is always less than that normally present in air and in fact it may be entirely absent due to its removal by the contents and the metal of the container. The volume of air originally present, however, can be computed on the basis of the amount of nitrogen found and, if the headspace volume is known, the original vacuum can be calculated. The gas can be collected by displacement under water, the amount of water drawn into the can representing the headspace. Care must be taken

to remove all the gas by tilting the can and by pressing the can ends.

Over-filling. Due to expansion of the contents, over-filled cans become strained during retorting. The absence of vacuum in such cans results in flipping or, in severe cases, springing of the ends.

Panelling. This condition usually arises in the larger sized cans in which there is a very high vacuum, the bodies of the cans being forced inwards by atmospheric pressure. A similar effect is produced in pressure-cooled cans where the air pressure has been excessively high. In severe cases panelling may result in seam leakage, especially if the seam is of sub-standard quality but panelling is not normally regarded as a spoilage condition.

Rust. The classification of cans showing external rust formation requires very careful consideration. If, after removal of the rust, inspection with a hand lens reveals that the iron plate is definitely pitted it is advisable to classify such cans as spoiled since the danger of perforation is great. Cans which are slightly rusty without noticeable pitting of the iron may be considered fit for immediate sale and consumption. The application of a lacquer film will minimise rust formation and cans intended for long storage or exposure to severe climatic conditions (*e.g.*, products for export) are lacquered externally as a routine measure. Rust formation is particularly liable to occur under labels when the label adhesive contains hygroscopic substances.

Damage. Apart from other considerations, considerable bacteriological significance should be attached to cans damaged by rough handling. The important points where such cans are concerned are the extent and location of the damage. Marked deformation of the seam will be attended by considerable risk of leakage and cans with this type of damage should be classified as spoiled. Similarly, where dents on the can body are so severe that seam distortion has occurred, cans should be rejected, but slight indentations

on the can body are permissible. A seam of normal mechanical quality can sustain slight indentations without leaking and the effects of slight damage to the seam should therefore, be considered in conjunction with the quality of the seam.

REFERENCES

CAMERON, E. J. 1938. *Food Res.*, **3**, 91.
CROSSLEY, E. L. 1938. *J. Hyg.*, **38**, 205.
HIRST, F., and ADAM, W. B. 1937. Univ. Bristol Res. Sta., Campden, Monograph 1.
HOAR, T. P. 1936. *Tech Publ.* Series A, No. 30. Internat. Tin Research and Development Council, London.
JENSEN, L. B., WOOD, I. H., and JANSEN, C. E. 1934. *Ind. Eng. Chem.*, **26**, 1118.
MCCLUNG, L. S., and WHEATON, E. 1936. *Food Res.*, **1**, 307.
MORRIS, T. N., and BRYAN, J. M. 1931. Food Invest. Bd. Dept. Sci. Ind. Res., London. Spec. Rept. No. 40.
MORRIS, T. N. 1933. "Principles of Fruit Preservation." Chapman and Hall Ltd., London.
SCOTT, G. C. 1937. *Canning Age*, **18**, 190.
YESAIR, J., and WILLIAMS, O. B. 1942. *Food Res.*, **7**, 118.

CHAPTER VII

BACTERIAL FOOD-POISONING

BEFORE the true *rôle* of bacteria in food-poisoning was properly appreciated, illness resulting from the consumption of food was attributed to the presence of ptomaines or toxic amines which were believed to have been formed as the result of the bacterial decomposition of protein. This theory is untenable. It has been shown that before these products are formed in food, it reaches such an advanced state of putrefaction that it would be abhorrent to normal persons. Furthermore, it has been demonstrated by Savage (1931) and others that these protein degradation products are only toxic when tested in quantities far greater than those which would be ingested under normal circumstances. There is no real evidence that such products in food material are harmful and Tanner (1933) has pointed out that the " putrid poison " of the earlier workers may be known to-day as the toxins of *Clostridium botulinum* or *Staphylococcus* or *Salmonella* species. Recent evidence indicates that to these may possibly be added certain other organisms.

Food-poisoning of bacterial origin arises through the consumption of food infected with certain types of living organisms or of food in which bacteria have developed and produced toxin. In other words, food-poisoning may be an infection or an intoxication. Three types of organisms are recognised as the chief casual agents in bacterial food poisoning. These are *Clostridium botulinum,* certain organisms of the *Salmonella* group and certain types of staphylococci. In addition, accumulated evidence indicates that a variety of other organisms may produce toxic substances which, when consumed, give rise to illness.

Clostridium botulinum. This organism, which is responsible for the disease known as botulism, is widely distributed

in nature, having been isolated from virgin and cultivated soil in America, Europe, India, China and other parts of the world. Meyer and Dubovsky (1922) found it to be present in 9 out of 64 samples of soil collected from various English counties. Leighton and Buxton (1928) detected the organism in four samples of Scottish soil. During its growth in food materials *Clostridium botulinum* liberates an extremely potent exotoxin which attacks the nervous system, often with fatal results. Symptoms, which are highly characteristic, usually appear within 24 hours of consuming the food but the incubation period may be extended to 72 hours. Gastro-intestinal disturbance is rare. Most animals are susceptible to botulinus toxin and various diseases such as forage poisoning in horses and cattle and limberneck in fowls are due to the ingestion by the animals of feeding-stuffs intoxicated by *Clostridium botulinum*.

The organism is a saprophyte, incapable of development in the body and consequently does not cause infection. It is an obligate spore-forming anaerobe of the putrefactive type. Usually, botulinum-intoxicated food is obviously unsound, the indications including gas evolution, disintegration and rancid odour but there is abundant evidence that the food does not of necessity show evidence of spoilage.

Five types of *Clostridium botulinum* are recognised and these are designated A, B, C, D and E. Types A and B are those associated with botulism in man, A being encountered frequently. Each type is antigenically distinct and antitoxin prepared for one is inactive against the toxins of other types. The heat stability of the toxin varies with the different types but 15–30 minutes exposure at 80 deg. C. (176 deg. F.) is sufficient to destroy the toxins of types A, B and C. As stated earlier, the spores of *Clostridium botulinum* show considerable resistance to heat and the importance which attaches to the organism in relation to its growth in canned foods has led to the adoption of its thermal death-time as a minimum standard of processing for foods with

*p*H values above 4·5. For further details of botulism, Tanner's "Food Borne Infections and Intoxications" (1933) should be consulted.

Salmonella Group. Food-poisoning in man is most frequently due to certain types of the *Salmonella* group. These organisms are motile, non-sporing rods of low heat-resistance. All are Gram-negative and saccharolytic. As a group the *Salmonella* species are very closely related, in some cases their cultural and biological characteristics are so similar that differentation is only possible with the aid of complicated serological methods. *Salmonella* organisms of the food-poisoning type are frequently associated with disease processes in cattle and pigs. Rats and mice are also subject to epidemics due to certain Salmonella and human carriers not suffering from illness may harbour the organisms in their intestines.

In *Salmonella* food-poisoning, the food concerned is usually meat, milk, fish and eggs; especially have those foods such as prepared meats, sausages, and meat pies which are lightly cooked and subject to much manipulation been involved. The appearance and taste of infected food may not be noticeably altered.

The *Salmonella* types most commonly encountered as food-poisoning agents in this country are *Salmonella typhimurium* (also known as *S. œrtrycke*) and *Salmonella enteritidis*. Other *Salmonella* types which have been involved include *Salmonella choleræsuis* (var. *Kunzendorf*), *Salmonella sp.* (*Thompson* type). *Salmonella sp.* (*Newport* type), *Salmonella enteritidis* (var. *Dublin*) and *Salmonella sp.* (*Potsdam* type). Organisms of the dysentery group have also been noted in food-poisoning of the infection type.

The *Salmonella* organisms do not elaborate a filterable exotoxin but produce endotoxin and when living organisms of the food-poisoning type are ingested, acute gastro-enteritis results, usually after a 6–24 hour incubation period. The illness is not often fatal, but may persist for weeks.

There is much conflicting evidence in the literature regarding the ability of the *Salmonella* group to cause intoxication of the gastro-enteritis type. Much of the disagreement appears to have arisen as a result of the different methods adopted by the various investigators, some results being deduced from the results of feeding tests on animals while others have been based on the reactions following injection of material.

Savage and White (1925) and others have reported that heat-killed cultures of these organisms produced gastro-intestinal disturbance when administered to animals. The toxic substance was shown to be remarkably heat stable, remaining potent after 30 minutes heating at 100 deg. C. (212 deg. F.) and it was suggested that these irritant products were responsible for outbreaks of food-poisoning in which living bacilli were not found. This theory had particular reference to poisoning associated with canned foods in which it was believed that the living bacteria, but not the irritant substance, had been destroyed by heat-processing. Many of the experiments in which these heat-stable irritant substances were demonstrated were carried out by the injection of animals, a method open to criticism and although some of the investigations in which feeding tests were used gave positive results, these have not been generally confirmed. Dack, Cary and Harmon (1928) fed human subjects with heat-killed cultures of *Salmonella ærtrycke* and *Salmonella enteritidis* but failed to produce any symptoms of illness. Verder and Sutton (1933) also failed to incite illness in human volunteers with custards which had been infected with *Salmonella enteriditis* and heat-sterilised after incubation. In a short review of the subject Dack and Davison (1938) conclude that the evidence at the present time strongly indicates that *Salmonella* food-poisoning is due to infection with living organisms and not to the effects of pre-formed endotoxin.

Staphylococci. The incrimination of staphylococci as agents of food-poisoning is a comparatively recent develop-

ment. These free-growing organisms are facultative anaerobes; they are Gram-positive, non-motile and do not form spores and hence are relatively susceptible to heat-destruction. They are extremely widespread, being found in air, water, milk and sewage but their main source is the animal body where they are normally present on the skin and in the intestinal and respiratory tracts. During their growth on food materials, certain strains of staphylococci elaborate a filtrable enterotoxic substance which gives rise to acute intestinal disturbance. The enterotoxic substance is distinct from other toxins produced by the organisms and there is some evidence that it is a metabolite and not a true toxin (Eaton, 1938). The incubation period following ingestion of intoxicated food is usually 2–4 hours and while the symptoms may be severe, recovery is rapid following elimination of the irritant from the body. As with other types of food-poisoning, the affected food may be quite normal in appearance and taste.

The majority of the outbreaks in which staphylocci have been concerned have occurred with " prepared " or unheated foods such as custard-filled pastries, milk, cheese, salads, ice-cream and sandwiches. Jordan and Hall (1931) reported an outbreak due to canned chicken and Minett (1938) recorded another involving potted meat paste. In the writer's experience a canned fish product was implicated when several members of a family suffered from gastro-intestinal symptoms. From the remains of the contents of the can a profuse growth of *Staphylococcus aureus* was obtained. All the indications were that the fish was the vehicle for the causal agent; it was the only food taken by all the persons affected. There was reason to believe that the can had become infected after processing.

Apparently no special types of staphylococci are concerned in the production of enterotoxin; Striter and Jordan (1935) state that the ability to cause gastro-intestinal disturbance is not confined to any recognisable type. The

identification of staphylococci as food-poisoning types cannot be carried out by ordinary methods, the greatest difficulty being the absence of a simple laboratory technique for demonstrating the enterotoxin. The usual laboratory animals are not susceptible when fed. Stone (1935) developed a cultural method for the identification of food-poisoning types but the claims for this medium have not been substantiated by Chinn (1936) and others. Jones and Lochhead (1939) claim that a pipette-feeding method, using kittens, possesses advantages over other methods. The use of human volunteers appears to be the most reliable test.

The staphylococci are relatively susceptible to heat and do not constitute a problem in the heat-processing of canned foods but there is some controversy concerning the heat-stability of the enterotoxin. Dolman, Wilson and Cockcroft's (1936) experiments indicated that when tested by the injection of kittens, staphylococcal enterotoxin was not inactivated by 30 minutes heating at 100 deg. C. (212 deg. F.). Jones and Lochhead (1939) have criticised this finding on the grounds that the injection method of testing is unreliable. In this connection, Rigdon (1938) has reported that typical symptoms were produced by the injection of kittens and puppies with sterile media containing no toxin. The feeding tests carried out by Jones and Lochhead indicated that staphylococcal enterotoxin was destroyed or markedly reduced by boiling for 30 minutes. In a recent publication, Laing and Shinn (1940) have concluded that the heat processing conditions which suffice for the destruction of *Clostridium botulinum* spores are more than adequate for the inactivation of staphylococcal enterotoxin although it should be remarked that their results, based on monkey feeding tests, indicated a considerable heat stability of the enterotoxin. These authors consider that canned foods are beyond suspicion as vehicles in the transmission of staphylococcal food-poisoning. The data presented by Laing and Shinn is given in the following table :—

Temperature (approx.) °F.	Maximum Survival Time Spores of *Cl. botulinum*	Minimum Destruction Time (Ideal) Spores of *Cl. botulinum.*	Negative Reaction Monkeys fed Heated Toxic Filtrates.
	(Minutes)	(Minutes)	(Minutes)
240	10	12	6
230	33	36	12
220	100	106	30
212	330	360	120

Miscellaneous Types. Topley and Wilson (1936) state that the evidence accumulated during recent years indicates that gastro-enterotoxic substances may be formed in food materials by organisms which are not normally associated with illness. According to these authors—"The pendulum is in fact slowly swinging in the direction of the old 'ptomaine' theory, with the distinction that the poisons now regarded as responsible are not the result of advanced protein decomposition, but are products of the growth in the food of those bacteria which are able to proliferate enormously without greatly altering the appearance and taste of the food."

In addition to *Clostridium botulinum* and the *Salmonella* and *Staphylococcus* groups, the bacterial types which have been reported as the agents of food-poisoning include *Escherichia coli*, *Proteus* and *Streptococcus* species. That these and other organisms can, under certain conditions, elaborate gastro-enterotoxic substances was shown by Jordan and Burrows (1935) who reported that various strains produced gastro-intestinal poison after repeated transfer on a starch medium in an atmosphere containing 20 per cent. carbon dioxide.

Whilst in some cases the "non-pathogenic" organisms have been incriminated largely on the basis of clinical evidence, the bacteriological findings in many recent cases appear to be convincing. Copper, Davis and Wiseman (1941) reported an outbreak due to brawn infected with

Proteus species. These organisms were found to produce an enterotoxin which was active for kittens. Most of the cases reported in which " non-pathogenic " organisms were involved have been of the " toxin " type but in Cary, Dack and Davison's (1938) experiments food-poisoning of the infection type was observed with streptococci.

Relation of Food-poisoning to Canned Foods

Considering all the evidence available and having due regard to the efficiency of modern processing conditions, commercially canned and preserved foods are of minor significance as vehicles for food-poisoning. Many of the cases in which canned foods have been concerned appear to have been based on clinical rather than bacteriological findings. In the past considerable prejudice has existed concerning the public health aspects of canned foods and to some extent this prejudice continues among a very small section of the population but it is an incontestable fact that canned foods are less liable than fresh foods to give rise to food-poisoning.

In Great Britain there is no record that canned food has ever been incriminated in botulism outbreaks and only on two occasions have glass-packed foods been involved (Savage, 1939). Due to the widespread usage of inadequately processed home-canned foods, the incidence of botulism cases is higher in America but Tanner, Beamer and Rickher (1940) point out that no authenticated case of botulism involving commercially canned food has occurred in that country since 1925. Tanner's (1933) statement that " the spectacular symptoms, the suddenness with which the symptoms appear and the high death rate combine to give botulism a place quite out of proportion to its significance as a cause of death," would appear to have special point where canned foods are concerned.

Savage (1939) has reviewed the question of canned foods

in relation to health and has summarised data concerning food-poisoning outbreaks and the foods involved. Of 121 outbreaks in Great Britain traceable to living *Salmonella* organisms during the period 1919 to 1931, canned foods were implicated on eight occasions. In the same period 58 out of 70 " toxin " type outbreaks were associated with canned foods. During the period 1932–1937 canned foods were the vehicle for 54 out of 429 outbreaks of food-poisoning, 51 being of the " toxin " type.

Staphylococcal food-poisoning is rarely associated with canned foods. The low heat resistance of these organisms, coupled with the effect of modern processing conditions on the enterotoxin renders these organisms theoretically insignificant in this connection. In the problem of post-process leakage, however, non-gas forming organisms such as staphylococci and streptococci may assume a greater significance in relation to canned food-poisoning. The ubiquity of these organisms makes it possible that occasional cans may become infected and intoxicated without apparent signs of spoilage. According to Davison and Dack (1942), commercial experience is that contamination after processing involves gaseous spoilage. While it is true that in the normal way, the majority of leaky cans are eliminated as " swells " it seems unreasonable to assume that gas-forming bacteria must of necessity be present among the invading bacteria. If staphylococcal or streptococcal species effect entrance unaccompanied by gas-forming bacteria the can will not " swell " and the contents may appear normal in spite of extensive development of the organisms. In view of the ability of organisms of this type to form enterotoxic substances, it seems possible that some of the earlier cases of canned food-poisoning, where, in the absence of living organisms of the *Salmonella* group, the causal agent was considered to be heat stable toxin, may have been due to contamination with such organisms as staphylococci and streptococci after processing.

Food Idiosyncrasy

The consumption of certain foods induces illness in persons who are hyper-sensitive to protein contained in the food, which, in normal persons, is without effect. The symptoms produced may be of a severe nature and are often similar to those occurring in bacterial food-poisoning with the result that illness following a meal is sometimes mistakenly attributed to unsoundness of the food. Among the foods which give rise to illness in hypersensitive persons are fish, especially shell-fish, eggs, cheese, tomatoes and strawberries but almost any protein-containing food may be responsible. The reactions whereby the symptoms are produced in susceptible subjects are related to immunological reactions involving antigen and antibody and are fully discussed by Topley and Wilson (1936).

REFERENCES

CARY, W. E., DACK, G. M., and DAVISON, E. 1938. *J. Infect. Dis.*, **62**, 88.

CHINN, B. D. 1936. *Food Res.*, **1**, 513.

COOPER, K. E., DAVIS, J., and WISEMAN, J. 1941. *J. Path. and Bact.*, **52**, 91.

DACK, G. M., CARY, W. E., and HARMON, P. H. 1928. *J. Prev. Med.*, **2**, 479.

DACK, G. M., and DAVISON, E. 1938. *Food Res.*, **3**, 347.

DAVISON, E., and DACK, G. M. 1942. *Food Res.*, **7**, 80.

DOLMAN, C. E., WILSON, R. J., and COCKCROFT, W. H. 1936. *Canad. Publ. Health J.*, **27**, 489.

EATON, M. D. 1938. *Bact. Reviews*, **2**, 1.

JONES, A. H., and LOCHHEAD, A. G. 1939. *Food Res.*, **4**, 203.

JORDAN, E. O., and HALL, J. R. 1931. *J. Prev. Med.*, **5**, 387.

JORDAN, E. O., and BURROWS, W. 1935. *J. Infect. Dis.*, **57**, 121.

LAING, M. L., and SHINN, B. M. 1940. *Canner*, **90**, 85.

LEIGHTON, G., and BUXTON, J. B. 1928. *J. Hyg.*, **28**, 79.

MEYER, K. F., and DUBOVSKY, B. J. 1922. *J. Infect. Dis.*, **31**, 600.

MINETT, F. C. 1938. *J. Hyg.*, **38**, 623.

RIGDON, R. H. 1938. *Proc. Soc. Exp. Biol. and Med.*, **38**, 82.

SAVAGE, W. G. 1921. *J. Hyg.*, **20**, 69.

SAVAGE, W. G., and WHITE, P. B. 1925. Medical Res. Council. Spec. Rep. Series No. 91.

SAVAGE, W. G. 1939. *Lancet*, **237**, 991.

STONE, R. V. 1935. *Proc. Soc. Exp. Biol. and Med.*, **33**, 185.

STRITER, J., and JORDAN, E. O. 1935. *J. Infect. Dis.*, **56**, 1.

TANNER, F. W. 1933. "Food Borne Infections and Intoxications." Twin City Printing Co., Illinois.

TANNER, F. W., BEAMER, P. R., and RICKHER, C. J. 1940. *Food Res.*, **5**, 323.

TOPLEY, W. W. C., and WILSON, G. S. 1936. "Principles of Bacteriology and Immunity." Arnold & Co., London.

VERDER, E., and SUTTON, C. 1933. *J. Infect. Dis.*, **53**, 262.

CHAPTER VIII

LABORATORY EXAMINATION OF CANNED FOODS

Culture Media

In the past a great variety of culture media have been used for the bacteriological examination of canned foods. A common practice has been the employment of special media prepared from the food material to be examined but as Esty and Stephenson (1925) and more recently, Cameron (1936) have pointed out, the use of such media is unnecessary for routine examination purposes. It is now generally agreed that the nutritional requirements of the micro-organisms likely to be encountered in canned foods can be adequately served by a relatively few, easily prepared media. It should, of course, be understood that these media are only intended for the primary isolation of spoilage or dormant organisms. The complete identification of an organism involves the use of the standard media generally employed for pure culture study. Details of these are to be found in the many text-books dealing with practical bacteriology.

Culture media for the primary isolation of micro-organisms from canned foods can be grouped into those used for low-acid and medium-acid foods and those for acid foods. It is thought that it will be found most convenient to list the media under the groups of spoilage organisms for which they are employed.

Media for Low-acid and Medium-acid Foods (above *p*H 4·5)

Thermophilic Bacteria. (1) "*Flat-sour*" *Group*: Comparative tests reported by Williams (1936) demonstrated that dextrose tryptone medium is the most suitable for cultivating this group. It is prepared as follows :—

Bacto-tryptone . . .	10 gm.
Dextrose	5 ,,
Bromocresol purple . .	0·04 ,,
Water	1,000 ml.

Mix the ingredients and steam until dissolved. Adjust the reaction to *p*H 6·8–7·0, filter and sterilise at 15 lb. pressure for 20 minutes. For solid medium, add 15 gm. of agar to the above ingredients.

In the liquid medium the production of acid from the dextrose by " flat-sour " organisms causes the colour to change from purple to yellow. On the agar medium, surface colonies of typical " flat-sour " organisms are about 2–5 mm. in diameter, have a characteristic opaque centre and are surrounded by a yellow zone. With those types which are weak acid-producers the yellow zone may not be apparent on agar while the liquid medium shows turbidity with little or no colour change.

(2) *Saccharolytic Anaerobes.* For the detection of this group, corn-liver medium has gained wide acceptance. It was devised by McClung and McCoy (1934) who demonstrated its superiority over other types of media. It has been recommended for the general cultivation of anaerobic bacteria. The medium is prepared as follows :—

One or 2 per cent. dried liver (tissue from liver broth medium dried at 55–60 deg. C. and finely ground) and 5 per cent. corn meal are steamed for one hour, cooled and tubed. The medium is rather viscous and requires care in sterilising. After sterilisation for 2 hours at 15 lb. per sq. in. the pressure must be reduced slowly to avoid blowing out the plugs. This medium does not require exhausting by heating before inoculation, and sealing or special anaerobic technique is unnecessary. Growth is evident through the appearance of gas bubbles which are held in the viscous medium and there may also be digestion and noticeable change in reaction.

A widely used medium for anaerobic thermophiles is liver broth (preparation given below). Since this medium is also used for mesophilic organisms its use for thermophilic types has obvious economic advantages.

(3) H_2S *Producing Anaerobes.* For the detection of thermophilic anaerobes producing hydrogen sulphide, Cameron (1938) recommends the following medium :—

Tryptone	10 gm
Sodium sulphite . . .	1 ,,
Agar	20 ,,
Water	1,000 ml.

No adjustment of *p*H is necessary. Before dispensing, a clean iron nail is placed in each tube. In this medium characteristic blackened spherical areas are formed by the "sulphide" spoilage organisms. No gas is observed. Saccharolytic thermophilic anaerobes may cause a general blackening in this medium but they are readily distinguished by the splitting of the agar which results from gas production.

Mesophilic Bacteria. (1) *Spore-forming anaerobes* : The medium employed for the detection of the spore-forming mesophilic anaerobes in canned foods should be suitable both for putrefactive and saccharolytic types. A medium which fulfils this requirement and one which is widely used in canned food examination is liver broth. It is prepared as follows :—

Boil 500 gm. of minced beef liver in 1,000 ml. of water for one hour. Adjust the reaction of the mixture to *p*H 7·0 and boil for a further 10 minutes. Strain through muslin and make up the volume to 1,000 ml. Add 10 gm. of peptone and 1 gm. of dipotassium phosphate and adjust the reaction to *p*H 7·0. When the medium is filled off, about $\frac{1}{2}$–1 in. of the liver particles is placed in the bottom of each tube and covered with the broth to a depth of an inch. Sterilise at 15 lb. pressure for 20 minutes. Before inoculation, this medium must be steamed for 20 minutes to remove dissolved

air. After cooling and inoculation the surface of the medium is layered with a sterile vaseline-paraffin wax mixture or sterile 3 per cent. plain agar (cooled to 50 deg. C.–122 deg. F.). Growth in this medium is apparent through gas production which causes the seal to rise or split. Putrefactive anaerobes also give rise to foul odours.

As already mentioned, corn-liver medium has been recommended for the cultivation of anaerobes generally but according to Crossley (1941) it seems to be less suited to the putrefactive types. For use in canned food examination, Crossley endeavoured to find a simple medium which did not require special anaerobic technique and in which growth was readily apparent. None of the common media met his requirements and he evolved a milk medium which is prepared as follows :—

Milk (skim milk is preferred) with 1 per cent. peptone and 0·01 per cent. bromocresol purple is tubed in 10 ml. quantities and sterilised by steaming for 20 minutes on four successive days. For the examination of meat and fish pastes about 2 gm. of the material are inoculated into each tube. For vegetable or dairy products 2 gm. of sterile meat or fish paste are first added aseptically to the milk-peptone medium and the cultures then prepared in the usual manner. Anaerobic technique is not required for this medium which the originator found to be of diagnostic value, the growth reactions obtained permitting a rough classification of bacterial types. This would seem to be a very valuable medium and will no doubt receive full attention from those engaged in the routine examination of canned foods.

(2) *Spore-forming Aerobes and other Bacteria.* For the routine isolation of spore-forming aerobic or facultative organisms dextrose tryptone broth or agar is suitable and its use is recommended by Cameron (1936). Other organisms such as the non-sporing aerobic or facultative species found in "leaker" spoilage also grow well on this medium. Standard nutrient broth or agar with the addition of 1 per

cent. dextrose may also be used. Nutrient broth may be prepared as follows :—

Meat extract (Lab. Lemco)	3 gm,
Peptone . . .	5 ,,
Water . . .	1,000 ml.

Dissolve ingredients by steaming. Adjust reaction to *p*H 7·2 and filter. Sterilise at 15 lb. pressure for 20 minutes ; 1·5 per cent. agar is incorporated for solid medium.

Media for Acid Foods (below *p*H 4·5)

(1) *Spore-forming Bacteria.* The significant organisms which fall under this heading are limited to the *Clostridium pasteurianum* and the "flat-sour" thermophilic types. Spoilage resulting from the activities of these organisms has so far only been prominent in America but it may be advisable to include cultures for these types in any search for spoilage or potential spoilage organisms in such products as the less acid canned fruits or tomato packs. For *Clostridium pasteurianum* types, liver broth should be used while dextrose tryptone broth is employed for the "flat-sour" organisms.

(2) *Non-sporing Bacteria.* For the detection of acidophilic bacteria, Esty and Stephenson (1925) recommended the use of tomato juice and nutrient broth in equal parts with the addition of 1 per cent. dextrose. Because of its acidity, this medium can be effectively sterilised by steam at atmospheric pressure. For a solid medium 3 per cent. agar is added and sterilisation carried out by steaming for one hour.

(3) *Moulds and Yeasts.* Linden (1936) recommends a malt-extract medium for yeasts and moulds. It is prepared as follows :—

Malt extract (Difco) .	100 gm.
Distilled water . .	1,000 ml.

Dissolve the powdered malt extract by steaming. Adjust

the reaction to *p*H 4·7 and cool to 50 deg. C. (122 deg. F.). Add slowly 100 ml. of a 5 per cent. suspension of Bentonite and mix vigorously. Hold at 50–75 deg. (122–167 deg. F.) for 30 minutes, then filter through paper till clear. Heat for 10 minutes at 15 lb. steam pressure and filter through paper to remove any precipitate. Sterilise at 10 lb. pressure for 15 minutes and cool rapidly. For plating purposes add 2 per cent. agar.

A very useful medium for the cultivation of yeasts, moulds and acidophilic bacteria is wort broth. It is prepared by diluting brewer's wort with three times its volume of nutrient broth. One per cent. dextrose is added and after adjustment of the reaction to *p*H 6·0, the medium is steamed for 30 minutes, filtered and sterilised at 10 lb. pressure for 20 minutes. If solid medium is required 2 per cent. agar should be added.

For the detection of osmophilic yeasts, Lochhead and Farrell (1930) used a honey medium, which is prepared as follows :—

Honey	800 gm
Peptone	1 ,,
K_2HPO_4	1 ,,
$MgSO_4$	0·5 ,,
Ammonium tartrate	0·5 ;,
NaCl	0·1 ,,
$CaCl_2$	0·1 ,,
Distilled water to	1,000 ml

Mix the ingredients, the honey being previously warmed. Sterilise at 15 lb. pressure for 20 minutes. The reaction of this medium is approximately *p*H 4·2.

Outline of Routine Examination

Canned foods are usually submitted for bacteriological examination for one of two purposes :—

(*a*) To test the adequacy of heat-processing conditions.

(*b*) To determine spoilage causes.

In connection with bacteriological tests on canned foods a point which frequently receives insufficient attention concerns the number of samples examined. For sterility test purposes a negative result on a single sample is without significance except for that particular sample since sterile cans may occur even in under-processed batches. Furthermore, it should be appreciated that negative bacteriological findings are not in themselves sufficient evidence for assuming that the process to which test samples have been submitted was commercially adequate because the conditions (*e.g.*, initial contamination) operating when the test samples were packed may change through various causes. A process can only be properly assessed from a consideration of heat-penetration data, process time and temperature conditions and the results of experimental inoculation of the cans with organisms of known resistance. It follows therefore, that sterility tests should mainly be the concern of the factory control laboratory where an adequate number of samples from each batch are available for testing. The cans examined should, of course, be free from obvious mechanical defects or damage. Inadequate sampling may also lead to erroneous conclusions concerning spoilage causes. For example, in a batch of cans which have spoiled as the result of under-processing there may occur a few cans which have also become infected through leakage. This is possible even when the seams are of normal quality and if such a can is taken as being representative of the batch, the presence of leakage organisms (cocci, non-sporing rods) may give rise to confusion. It would therefore be most injudicious to base any conclusion on the result of an examination of a single sample.

Before outlining the procedure for canned food examination it may be said that the technique adopted in opening the can and sampling the contents is largely a matter of personal choice. Any method is permissible provided the

risk of contamination is kept at a minimum. The general outline which follows is based on the recommendations laid down by Esty and Stephenson (1925), Tanner (1932) and others.

Preliminary Examination of Container. A thorough external examination of the container should first be made for obvious mechanical defects in the seams, perforations, rust, damage, " peaks " or other abnormalities which may be usefully correlated with the bacteriological findings.

Preliminary Incubation of Samples. Except in the case of swelled cans, all samples should be incubated before cultures are made. The organisms which survive heat-processing may be extremely few in number and the purpose of preliminary incubation is to stimulate multiplication of the survivors and thus increase the chance of securing them in the inoculum. In view of the fact that sub-lethal exposure to heat may cause dormancy of spores, the time allowed for incubation is important. Various periods, ranging from one to several weeks have been recommended, but it frequently occurs that the urgency of the examination is a controlling factor. Unless further samples are available for prolonged incubation tests, a period of one week at 37 deg. C. (98·6 deg. F.) is desirable for low and medium-acid products. It is inadvisable, in view of the tendency of thermophilic bacteria to undergo rapid autosterilisation, to incubate samples intended for culture for longer than a few days at 55 deg. C. (131 deg. F.). Acid products may usefully be given 10 days preliminary incubation at 25 deg. C. (75 deg. F.); they should not be incubated at 37 deg. C. (98·6 deg. F.) since the activity of the acid may be increased to a point where it offsets any stimulative effect which the increased temperature may have on the development of the organisms. A similar proviso is given for any product known to contain such substances such as sulphur dioxide.

Technique of Sampling. In the preparation of cultures from canned products the main points at which contamina-

tion may occur are during the opening of the container and the removal and transfer of the inoculum to the medium. The first essential is that all operations be conducted in a still, dust-free atmosphere. If a small room cannot be exclusively reserved for culture work, a part of the work bench should be enclosed by a large hood under which the manipulations can be carried out, thus minimising the risk of aerial contamination.

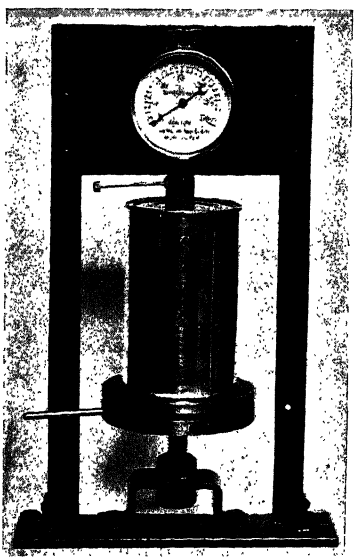


FIG. 8. Apparatus for aseptically measuring vacua in cans.

If dirty or greasy, the can end should first be thoroughly washed with hot soapy water. The end is then flooded with 2 per cent. sodium hypochlorite solution which is allowed to act for a minute or so. The germicide is then poured off and the can end gently flamed till dry and covered with a sterile petri dish. If knowledge of the vacuum in the can is desired, this may be obtained by means of a specially fitted vacuum gauge. The piercer of the gauge, which should be detachable, is fitted with a side arm packed with cotton wool and sealed by a screw-cap. The piercer, together with its rubber washer, is wrapped in stout paper and sterilised by autoclaving. In use, the sterilised piercer is screwed into position on the gauge and the previously sterilised can end held against the rubber washer. By screwing up the base plate the can is pierced and the vacuum noted. The cap at the end of the side arm is then released and the vacuum in the can neutralised by

filtered air entering through the side arm. The apparatus is shown in Fig. 8.

The can may be opened for sampling in a variety of ways. One method which has proved quite satisfactory is to puncture a large hole (at least $\frac{1}{2}$ inch wide) in the can end by means of a piercing tool as described by

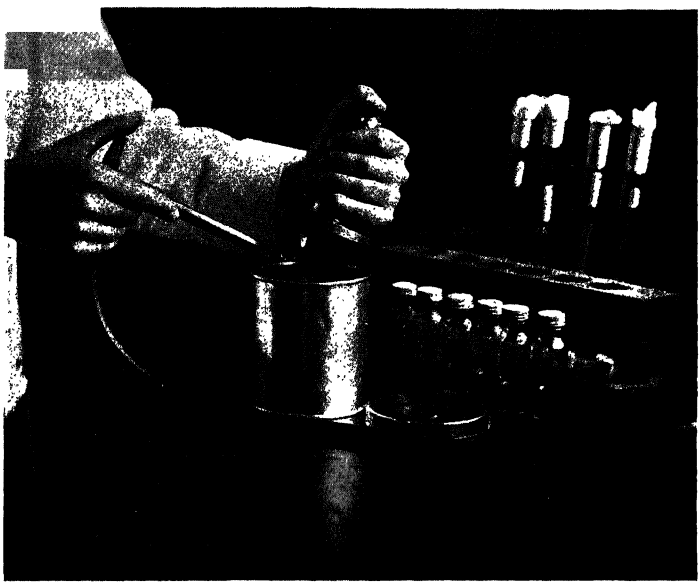


FIG. 9.

Bashford (1941). The tool consists of a steel rod about $\frac{1}{2}$–$\frac{3}{4}$ inch diameter and 9 inches long sharpened to a point at one end. About 2 inches from the tip a circular flanged shield resembling a petri dish is welded on. This affords some protection against contamination of the sample. The piercing tool is sterilised by flaming before use. When the puncture is made, the air replacing the vacuum in the can (assuming the vacuum test has not been

made) is sterilised by directing a Bunsen flame on the point of the tool (Fig. 9).

The method of removing the inoculum and the site from which it is taken depends on the nature of the product. With solid packs, a cylinder is removed with a sterile cork borer which is firmly plugged at the top with cotton wool. A rod to expel the sample from the borer is necessary. A fresh, sterile borer is required for each can but the expelling rod may be used repeatedly since it is prevented from making contact with the sample by the sterile cotton plug in the borer. In sterility tests on solid packs the inoculum is taken from the centre, since this portion receives least heat-treatment and viable organisms are, therefore, most likely to be found at this site. The sample is expelled from the borer into a screw-capped bottle containing about 50 ml. of sterile water and some chips of broken glass. At least 25 gm. of the sample is broken up by vigorous shaking and the suspension dispensed by means of a sterile pipette into the appropriate media, using about 2 ml. per 10 ml. of media. Bottles of about 100 ml. capacity have been found very useful and are superior to flasks since they can be shaken more vigorously. When examining solid packs for spoilage, it is important that the sample taken should include material from the exterior of the can contents; whereas in sterility tests the organisms are most likely to be found in the centre of the product, they will probably be confined to the surface if the can has leaked for any reason. In such cases, therefore, sampling should be carried out by pushing the borer vertically and obliquely in several directions to make contact with the container, thus ensuring that material is obtained from the surfaces of the contents.

With liquid products or those in which there are solid and liquid constituents, the liquid portion should be sampled, using large (20 ml.) pipettes and inoculated direct into the media. The bore at the tips of the pipettes should not be too narrow. For semi-solid or viscous products lengths of

untapered glass tubing (4 mm. bore) are convenient for sampling.

When a large number of samples have to be examined, as in routine sterility tests, it may be necessary to modify slightly the above methods which in these circumstances would necessitate the use of a large number of sterile petri

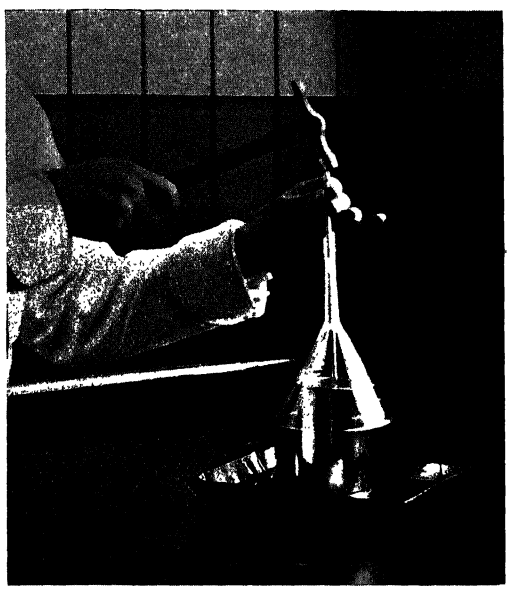


FIG. 10.

dishers and borers. As substitutes for petri dishes, sterilised circles of tinplate may be used for protecting the can ends after sterilisation. With solid products, the can is punctured in the manner stated ; the can is then opened with a flame-sterilised can opener of the blade cutter type, care being taken to avoid damaging the seam. The top portion of the contents are removed with a flamed knife and a sample from the centre is taken with a flamed spatula, dispersed

in water and inoculated into the various media. Liquid products do not present any problem since it is usually possible to maintain an ample stock of sterile glass pipettes or tubing.

Technique for Sampling Swelled Cans. When sampling swelled cans some precaution must be taken to minimise the spurting of the contents. An apparatus giving excellent protection to the operator consists of a large glass filter funnel, firmly plugged in the stem with cotton wool through which is passed a thin steel rod with a sharpened tip. The whole apparatus is wrapped in stout brown paper and sterilised under steam pressure. In use, the funnel is inverted over the previously cleaned and sterilised can, the sharp tip of the rod resting on the can end (see Fig. 10). A blow on the blunt end of the rod forces the tip through the can, the funnel preventing the spurting contents from contaminating the operator. The can should stand in a shallow tray of dilute hypochlorite to receive any material which may drip from the funnel. When the pressure has subsided the inoculum is withdrawn in the usual way.

Preparation of Cultures. The cultures prepared and the choice of media will depend on the object of the investigation and the type of product concerned. In certain circumstances it is advisable to prepare a number of each type of culture, as for example, when the preliminary incubation period has been unavoidably shortened and the possibility exists that organisms may not be secured in a single culture.

The following system of cultures may serve as a guide :—

NON-ACID AND MEDIUM-ACID FOODS

Media	Incubation Conditions
Dextrose tryptone broth .	Aerobic, 37 deg. C. (98·6 deg. F.), 4 days.
Dextrose tryptone broth .	Aerobic, 55 deg. C. (131 deg. F.), 4 days.
Liver broth . . .	Anaerobic, 37 deg. C. (98·6 deg. F.), 4 days.
Liver broth . . .	Anaerobic, 55 deg. C. (131 deg. F.), 4 days.

Cultures for thermophilic organisms should be inspected daily and removed for further examination as soon as growth is evident since the organisms tend to die out rapidly.

In spoilage investigations, additional cultures should be prepared in dextrose tryptone broth and on agar, and these are incubated at 25 deg. C. (77 deg. F.) for five days in order to detect organisms originating from cooling water or air.

Acid Foods

Media	Incubation Conditions
Tomato dextrose broth .	Aerobic, 25–30 deg. C. (77–86 deg. F), 7 days.
Malt-extract broth or Wort broth.	Aerobic, 25–30 deg. C. (77–86 deg. F.), 7 days.
Dextrose tryptone broth .	Aerobic, 37 deg. C. (98·6 deg. F.), 4 days.
Dextrose tryptone broth .	Aerobic, 55 deg. C. (131 deg. F.), 4 days.
Liver broth . . .	Anaerobic, 37 deg. C. (98·6 deg. F.), 4 days.

Micro-examination of Contents. After preparation of the cultures smears of the contents stained by Gram's method are examined microscopically. This examination may serve as a check on the results obtained from the culture tests but it should be noted that correct interpretation of the microscopic evidence is sometimes difficult. It has already been mentioned that normal unspoiled cans may contain large numbers of non-viable organisms and that this condition may be due to the poor hygienic quality of the raw materials or to development of organisms during the period between the preparation of the food and heat-processing. Occasionally, cultures prepared from spoiled cans are sterile although, microscopically, large numbers of organisms may be visible in the contents. Such cases are due to autosterilisation of organisms which usually stain poorly or unevenly and show other signs of degeneration. Positive cultures are not often obtained from incubated cans which show no organisms in

direct smears unless the product contains substances inhibitory to growth, as for example, curing salts.

Hydrogen-ion Concentration. The reaction of the can contents should be determined particularly where the presence of " flat-sour " organisms is suspected. For comparison it is necessary that the normal *p*H of the food be known.

Detailed Examination of Container. Finally, a complete examination of the container should be made. An outline of the procedure for stripping can seams is given later.

Examination of Glass Packs. The bacteriological examination of heat-preserved foods packed in glass is made on lines similar to that for canned foods. Sampling of these products is simplified as the metal cap is easily removed but precautions must be taken in neutralising the vacuum. To avoid disturbing the gasket, the position of which may have an important bearing on the results of the examination, the caps should be removed as carefully as possible. In the examination of the container attention is directed to the following points which are the main causes of leakage :—

(1) Defects or chipping of the glass rim resulting in imperfect seating of the rubber gasket.

(2) Incorrect placing of the paper liner so that it laps over the rim of the glass, thus preventing proper seating of the gasket.

(3) Twisting of the gasket.

(4) Perforation of the metal cap.

Incubation Tests (Cans and Glass Packs). Culture tests should always be augmented by the incubation of additional samples. Low-acid and medium-acid products are held at 37 deg. C. (98·6 deg. F.) and 55 deg. C. (131 deg. F.) for at least 30 and 10 days respectively. At the end of the incubation period cans should be cooled to room temperature and vacuum and *p*H determinations made. Products susceptible to " flat-souring " may also be stored at 25 deg. C. (77 deg. F.) for 3 months. From *p*H determinations of samples so stored

the "flat-souring" activity of the organisms at normal storage temperatures can be assessed.

Acid products should be stored at a temperature of 25 deg. C. (77 deg. F.) for a minimum period of 4 weeks after which the vacuum is determined. Canned or bottled fruits are examined for signs of spoilage by *Byssochlamys fulva* or other organisms. Acid sauces, ketchups, pickles, etc., should be examined for evidence of fermentation. Gaseous spoilage in glass packs of this type is frequently not apparent until the closures are released, due to the fact that the gas is held in solution under pressure.

Apart from the final examination it is of course imperative that frequent inspection of samples be made throughout the incubation period and that samples be removed for detailed examination as soon as spoilage is apparent.

Dilution Cultures

In addition to the heat-preserved packs many canners produce lines which are preserved with edible acids, salt, sugar or combinations of these. In some cases these products, which include salad cream, sauces, pickles, etc., may be given a moderate heat treatment, but invariably they are unsterile. Especially where heating is not applied, the contaminating organisms may include bacteria, yeasts and moulds. The main problem is to decide whether the organisms present will cause spoilage. Demonstration of growth in a nutrient medium will not be of much assistance in this respect except in so far as to indicate the presence of possible spoilage types. A procedure which has been found useful involves the graded dilution of the product with a suitable nutrient medium. For example, salad cream known to be contaminated with yeast or aciduric bacteria may be diluted 5, 10, 20 and 50 per cent. with wort broth and held at 25 deg. C. (77 deg. F.). If fermentation occurs in the lowest dilution it may be deduced that there is little margin of safety with regard to the product's resistance to spoilage. If con-

siderable dilution is necessary before fermentation occurs it may be inferred that the product is reasonably resistant to spoilage. An alternative method has also proved useful:—

Suitable dilutions of the product are inoculated with a significant organism and the results of periodic counts of the viable organisms in each mixture are plotted. Useful information can be deduced from the curves so obtained.

Examination of Raw Materials for Thermophilic Spores

Sugar. The following procedure for the detection of thermophilic bacteria in sugar is that recommended by Cameron (1936a).

Sampling. From each delivery, ½-lb. samples are taken from each of five bags.

Place 20 gm. of sugar in a sterile 150 ml. flask marked to indicate a volume of 100 ml. Add sterile water to make up to 100 ml. The solution is brought to the boil and boiling continued for 5 minutes. Loss due to evaporation is replaced with sterile water.

" *Flat-Sour* " *Spores.* Into five petri dishes, pipette 2 ml. of boiled sugar solution. Cover with dextrose tryptone agar and mix thoroughly. " Spreaders " may be inhibited by covering the cooled dextrose tryptone media with a thin layer of 2 per cent. plain agar. The plates are incubated at 55 deg. C. (131 deg. F.) for 36–48 hours. The total number of typical acid-producing colonies for the five plates is determined and this number indicates the " flat-sour " spores in 2 gm. of original sugar. This number is multiplied by 5 to give the spore content of 10 gm. of sugar. Non-acid types of colonies are not included in this count.

Standard. For the five samples examined there should be a maximum of 75 spores and an average of not more than 50 spores per 10 gm.

Total Thermophilic Spores. This count includes the non-acid in addition to the acid-producing colonies.

Standard. For the five samples examined there shall be

a maximum of 150 spores and an average of not more than 125 spores per 10 gm.

Anaerobic Spores (not producing H_2S). Twenty ml. of the boiled solution are divided in roughly equal amounts between six liver broth tubes. These are stratified with plain nutrient agar or wax and incubated at 55 deg. C. (131 deg. F.) for 72 hours. Results are expressed as the number of tubes positive or negative, *e.g.*, $+++---$.

Standard. Spores of this type are permissible in not more than three of the five samples and in any one sample to the extent of not more than four tubes.

Sulphide Spoilage Spores. Twenty ml. of boiled solution are divided in roughly equal amounts between six tubes of melted sulphite agar. After thorough mixing the medium is allowed to set and the tubes are incubated at 55 deg. C. (131 deg. F.) for 72 hours. The typical black colonies are counted and the results expressed as the number of spores per 10 gm.

Standard. Spores of this type are permissible in not more than two of the five samples and in any one sample to the extent of not more than 5 spores per 10 gm.

Starches. For the detection of thermophilic spores in starch, Clark and Tanner (1937) have proposed modifications of Cameron's method for sugar. They state that a longer heating period is necessary for the destruction of non-spoilage organisms in starch than in sugar and accordingly a heating period of 30 minutes is recommended. This method is as follows :—

Preparation of Samples. Ten gm. of carefully mixed starch are weighed into a sterile flask. To this is added, with stirring, enough sterile water to make up to 100 gm. This mixture is then thoroughly stirred and pipetted into the media used for detecting the various groups of spoilage organisms.

Flat-Sour Spores. Twenty ml. of the prepared starch suspension are added to 100 ml. of melted tryptone bromo-

cresol-purple agar, the mixture being stirred as the starch suspension is added. This mixture is then heated with shaking until the starch flows and is then steamed for 30 minutes with occasional stirring. It is then distributed equally among six petri dishes. After the medium has set the plates are incubated at 55 deg. C. (131 deg. F.) for 36–48 hours.

Anaerobic Spores (*not producing* H_2S). Twenty ml. of the starch-water mixture are equally distributed among six tubes of liver broth previously heated to 100 deg. C. (212 deg. F.). After re-heating and agitation to distribute the starch thoroughly and prevent excess foaming, the tubes are heated at 100 deg. C. (212 deg. F.) for 30 minutes, cooled and stratified with plain agar. Incubate at 55 deg. C. (131 deg. F.) for 5 days.

Anaerobic Spores (*producing* H_2S). Twenty ml. of the starch-water mixture are distributed equally between six tubes of melted sulphite agar. After thorough mixing the tubes are heated at 100 deg. C. (212 deg. F.) for 30 minutes. After heating, the medium is again thoroughly stirred, allowed to harden and incubated at 55 deg. C. (131 deg. F.) for 5 days. The standards for starch are the same as those for sugar.

REFERENCES

BASHFORD, T. E. 1940. *Food Manuf.*, **15**, 181 and 276.
CAMERON, E. J. 1936. *J. Ass. Off. Agric. Chem.*, **19**, 433.
CAMERON, E. J. 1936a. *J. Ass. Off. Agric. Chem.*, **19**, 438.
CAMERON, E. J. 1938. *J. Ass. Off. Agric. Chem.*, **21**, 452.
CLARK, F. M., and TANNER, F. W. 1937. *Food Res.*, **2**, 27.
CROSSLEY, E. L. 1941. *J. Soc. Chem. Ind.*, **60**, 131.
ESTY, J. R., and STEPHENSON, A. E. 1925. *J. Infect. Dis.*, **36**, 486.
LINDEN, B. A. 1936. *J. Ass. Off. Agric. Chem.*, **19**, 440.
LOCHHEAD, A. G., and FARRELL, L. 1930. *Canad. J. Res.*, **3**, 51.
MCCLUNG, L. S., and MCCOY, E. 1934. *J. Bact.* **28**, 267.
TANNER, F. W. 1932. "Microbiology of Foods." Twin City Printing Co., Champaign, Illinois.
WILLIAMS, O. B. 1936. *Food Res.*, **1**, 217.

CHAPTER IX

EXAMINATION OF CAN SEAMS

THE mechanical condition of containers bears such an important relation to the bacteriological findings that seam examination must be regarded as an essential part of any investigation on canned foods.

It cannot be said that a wholly satisfactory method of detecting leaks in can seams has as yet been worked out. One method is to test the air-tightness of the can by means of compressed air while the can is held under water, leakage being indicated by the appearance of air bubbles. A serious objection to this method is that a can which has leaked (as shown by bacteriological tests) may subsequently become air-tight, the original leak being sealed by the can contents. Support for this view is derived from the fact that leakers frequently become hard swells, the defect in the seam being so effectively sealed that the pressure in the can builds up and may eventually cause it to burst. Another method is to judge the seam by inspection and measurement, using standards which are accepted as giving a reasonable degree of safety against leakage.

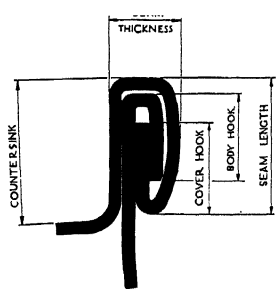
It should be appreciated that only the relatively gross defects are determinable by inspection and measurement and failure to establish the existence of mechanical defects must be anticipated in a proportion of spoiled cans when the bacteriological evidence clearly indicates leakage. A tolerance of five to ten thousandths of an inch is usually permissible in seam dimensions and it is not always sufficiently appreciated that since the average bacterium is less than one-five thousandth of an inch in length, it is possible for infection to take place through apparently sound seams, if, say for any reason, the lining compound is disturbed. Furthermore, this situation is aggravated when seams,

although within standard tolerance, are composed of thin tinplate. The incidence of such "non-apparent" leakers is raised by straining of can seams by excessive internal pressure resulting from too rapid reduction in steam pressure at the end of the retorting process. It will generally be found that such leakers are most prevalent in thin or watery products, probably for the reason that in the more viscous packs such as thick soups, any microscopic channels in the seams tend to be blocked by the solid material present.

A seam of normal commercial quality is expected to conform to certain standards of measurement. The standards generally accepted in England for the common sizes of "open-top" sanitary cans are :—

Seam length : not more than 0·125 inch.
Countersink : 0·125 inch.
Body hook : 0·075–0·085 inch. (outside length).
Cover hook : 0·075–0·085 inch. (outside length).

Seam dimensions are directly governed by the setting of the machine and the condition of its parts ; they are, in conjunction with other tests, extremely useful in controlling the seaming operation. They are to a certain extent dependent on the thickness of tinplate being reasonably constant and on the amount and even application of the lining compound but their value in estimating seam quality is undeniable.



DESIGNATION OF SEAM MEASUREMENTS

FIG. 11.

Significant seam dimensions are shown in Fig. 11. The measurements are made with a micrometer screw-gauge or with special seam rules which are generally obtainable from manufacturers of cans or can-seaming machinery.

Selection of Cans for Examination. It is important that

conclusions concerning a large number of swelled cans are not based upon the examination of only one or two samples. In any batch of canned foods a very small proportion of leakers must be anticipated, the normal level being something less than 1 per 1,000. These spoiled cans may arise from a multiplicity of causes or circumstances which are more or less uncontrollable ; undoubtedly a major factor is the impossibility of narrowing the mechanical tolerance for seams below the limits stated above. When the proportion of spoiled cans shows a marked increased over the normal level it is usually due to some relatively gross defect or defects in the process of can fabrication which can be detected by seam examination on the lines indicated here. It follows, therefore, that haphazard selection of a few samples from a batch of spoiled cans may give rise to inconclusive results if the cans chosen are mostly of the "non-apparent" leaker type. Unless the number is unduly large at least 10 per cent. of the samples involved should be examined in detail, after having ascertained the general features shown by the majority of the cans. When a large number of cans are involved, the detection of the defect can often be facilitated by the careful selection of cans for detailed examination. Generally, the most easily diagnosed cans are those showing moderate pressure with leakage, and if such cans form a fair proportion of the batch they should be selected for examination. It is, however, essential that any cans selected should show the same features as those present in the majority ; if the general feature is absence of apparent leakage, the cans selected should be of this type.

General Preliminary Examination. A careful visual examination of all cans should first be made. From this preliminary inspection experienced persons can detect abnormalities so that it may only be necessary to strip or section seams in order to confirm opinions formed as the result of the external examination. For example, a long can

seam invariably indicates a short cover hook and a narrow rounded seam is an indication of looseness. A small hand lens should be used for the examination.

To a certain extent visible leakage of the contents through any seam can be considered as being of diagnostic importance. As a general guide, it may be taken that if the ends of a "swell" can be "sprung" with moderate thumb pressure, a leak indicates a mechanical defect. On the other hand, if the internal pressure is so great that the can is a "hard swell" any leakage of the contents through the seam should be viewed with reserve, since with very high pressures the contents may be forced out, even through sound seams.

Examination of Rolled Seams. It is generally acknowledged by most authorities that a seam should be judged as a whole and not solely by its external dimensions or those of its separate parts. The external dimensions of the seam naturally vary slightly with the thickness of the tinplate and do not exactly indicate the relative position and size of the hooks. An examination of the hooks after separation likewise gives no indication of their exact relative position in the seam or the tightness of the seam as a whole.

1. *Contour.* First, examine the contour of the seam. Its outline should be free from sharp edges; the seam should not however, be beaded or rounded. Rounded seams are usually due to excessive rolling by the first operation wheel forming a strong curl which resists the subsequent pressure of the second operation wheel. The top of the seam should be distinctly but not excessively flattened. Sharp edges at the top inside of the seam known as "cut covers" are most frequently found on the cover where it coincides with the lock seam of the body. They may be caused by wearing or chipping of the chuck, incorrectly set second operation wheel and other maladjustments and the plate may be cut completely through with the result that leakage occurs.

2. "*Spurring.*" "Spurs" may result from failure of the

wheeling process to fold completely the cover hook under the body hook at one or more points. They appear as protrusions at the base of the seam (see Fig. 12). If sections with a fine jeweller's saw are made through the seam at the point where the "spur" occurs, it will be found that the cover hook is notched or in the case of large "spurs" it may be non-existent so that there is little or no engagement with the body hook. "Spurs" are a notable cause of leaker

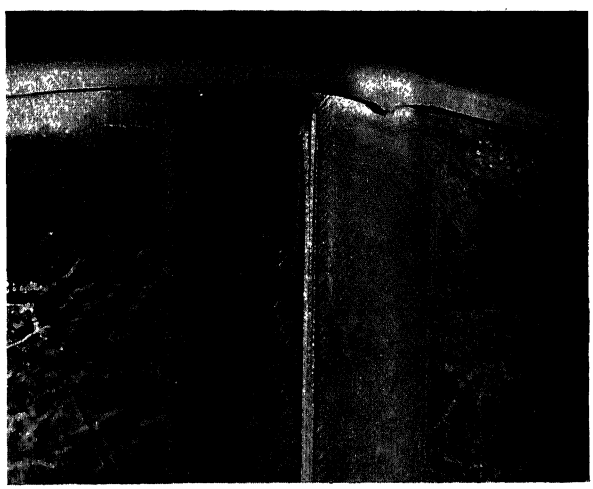


FIG. 12. Enlarged view of a "spur."

spoilage and they are most frequently observed at the overlap, *i.e.*, where the rolled seam coincides with the soldered side seam.

Among the causes of "spurring" may be mentioned harsh plate, worn seaming wheel bearings and pins, maladjustment of first and second operation wheels, incorrect base-plate pressure and irregular curl on can ends.

3. *Seam Thickness.* Due to the difficulty of obtaining tinplate of a constant thickness, there are no generally accepted standards for seam thickness. There should not,

however, be any marked variation in the thickness of any given seam apart from the normal increased thickness where the rolled seam coincides with the side seam. This increased thickness is due to the two extra thicknesses of metal where the overlap is hooked. Measurements should be taken at several points on the circumference and variations marked. With a tinplate of average thickness, say

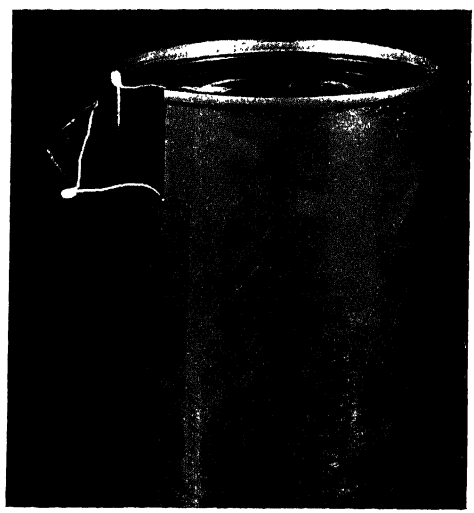


FIG. 13.

0·01 inch, the seam thickness should be in the region of 0·06 inch, a variation up to five-thousandths of an inch being permissible. If a triangular section (see Fig. 13) is made through the seam at the point of maximum thickness a looseness of closure which is not apparent in other parts of the seam can usually be observed. A hand lens should be employed in examining the section.

Variation in seam thickness may be due to worn chuck, grease or dirt on the base-plate causing the can to slip

during the seaming, second operation wheel too tight, worn machine bearings causing chuck to be off centre or uneven application of lining compound.

4. *Condition of Hooks.* Leakage of contents from several places in the rolled seam of a " soft swell " indicates defective engagement of the body and cover hooks. The lack of proper engagement may be due to one or both hooks being short

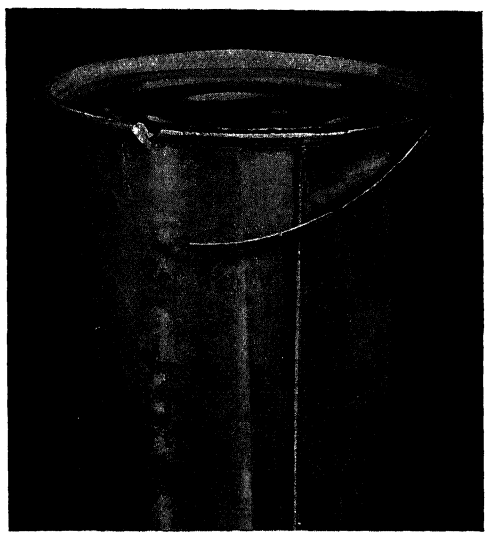


FIG. 14.

or the hooks may be of normal length but not flattened tightly together. Sections cut through the seam at the point of leakage will indicate whether the defect is due to short hooks or looseness. In the latter case the hooks appear curved or bowed in section and wrinkling is apparent when they are separated.

In order to separate the hooks for examination and measurement, first puncture the can to allow escape of gas. With the edge of a flat file cut a notch completely through

the seam. Starting from this notch and working round the whole circumference, file away the top outside edge of the seam until the second layer of metal (the body hook) is reached. The file should be held at an angle of about 45 deg. with the can body during the filing operation and care must be taken to avoid filing into the top of the body hook. Starting at the filed notch and tapping sharply with the flat face of the file on the cut edge of the seam, the cover hook is disengaged, leaving the body hook exposed (see Fig. 14). The tenacity with which the cover hook is held in place is an indication of the tightness of the seam.

The outside length of both hooks should be measured at several points. This is necessary because the length of a hook may vary at different places. The hooks should be free from wrinkles or folds. These indicate seam looseness ; on a properly flattened seam all wrinkles should be ironed out smooth by the second operation wheel.

The chief cause of body hook shortness is lack of base-plate pressure. It may also arise if there is too much clearance between seaming wheels and chuck ; other causes include short plate, *i.e.*, body blanks cut too narrow, and uneven flanging. It should be noted that the body hook at the overlap of the side seam is sometimes slightly short, the upper flap of metal tending to form an angle with the under flap at their lower edges. It is equally important that hooks should not be too long. Excessive base-plate pressure resulting in long body hook causes a corresponding decrease in the length of the cover hook and consequently affects the degree of engagement of the two hooks ; it also induces " spur " formation.

When the cover hook is short, this is usually due to failure of the first operation wheel to tuck the cover far enough under the flange of the body. Due to the fact that it has to be bent round two thicknesses of metal at this point, the cover hook is invariably slightly short at the overlap of the side seam. It seems highly probable that many cases

of leakage in which mechanical defects are not obvious arise through this " normal " shortening of the hooks at the side seam overlap.

Defective Soldering. Defective soldering of lock seams or of overlaps is almost always attended by leakage of the contents. Frequently, in cases of marked solder deficiency, the tinplate in the vicinity of the defect exhibits an area of sulphide staining, indicating that the contents have leaked during the retorting process. Occasionally, however, minute channels permit infection without leakage of contents so that it is unsafe to rely on visible external leakage in judging soldered seams. In order to separate the overlaps for examination, the rolled seams at the ends of the can must be taken apart. The body hook formed by the flaps is then levered straight with a screw driver or similar tool. By firmly gripping the body close to the edge of the inner flap with pliers, and pulling outwards, the flaps should come apart (see Fig. 15), If there is marked deficiency of solder the flaps separate easily, but considerable force may be required if they are well soldered. A common defect is the presence of a small unsoldered channel running through an otherwise well soldered flap into the end of the fold of the side seam.

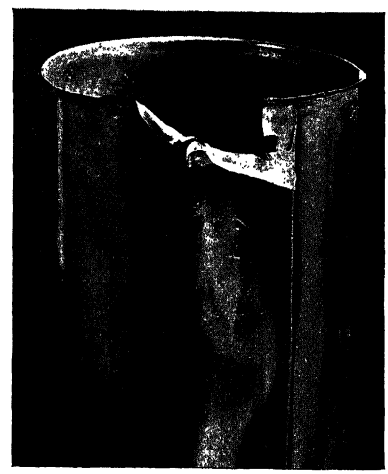


FIG. 15.

Side seams may be opened for inspection with the aid of an apparatus which consists of two semi-cylindrical lengths of metal which, when fitted together, form a cylinder of

slightly smaller diameter than the can. The can to be opened is slid over this cylinder and by operating a cam the cylinder is expanded until the side seam of the can is forced open. Before opening the side seam it is necessary to remove the cover hooks at both ends of the can. The opened seam should be carefully examined for solder-free patches with a hand lens.

Among causes of soldering defects may be mentioned springy or greasy tin-plate, poor fluxing, solder containing dirt or impurities, dirty solder roll, inefficient cooling fan, etc.

Fracture and Pin-holing of Plate. In addition to mechanical defects of seams, spoilage sometimes occurs through fractures or pin-holes in tin-plate. Unless the defect is very small it can usually be detected by leakage of the can contents but occasionally a minute fracture or hole may become blocked by the contents of the can and a "swell" may result. Minute fractures in tin-plate are sometimes caused by embossing code marks too heavily. Pin-holes frequently result from external rusting and spoiled cans showing rust formation require very careful inspection. After thorough cleansing with water and fine abrasive washing powder the corroded areas should be scrutinised with a hand lens.

Some of the common defects which result in "leaker" spoilage have been briefly mentioned; these are further summarised in the table which follows. "Leaker" spoilage is one of the outstanding problems confronting the canning industry, the aggregate annual loss due to this cause probably amounting to a very large sum. The minimisation of such losses undoubtedly depends upon the co-operative efforts of the engineer and the bacteriologist or canning technologist to ensure that cans with first-class seams are processed and cooled under ideal conditions.

Summary of some Common Seam Defects

Defects	Causes	Comments
Long seam . .	Insufficient 1st operation.	Cover hook is short—whole seam is stretched.
Short seam . .	Excessive 1st operation. Insufficient 2nd operation.	Sharp bend or "cut-over" at top inside edge of seam. Seam loose—hooks wrinkled.
Loose seam . .	Insufficient 2nd operation.	Seam thick and below normal length—hooks bow shaped and not tightly engaged—show wrinkling.
Uneven seam .	Worn rollers, roller pins on chuck spindle or other machine parts. Excessive 1st or 2nd operation.	Variation in seam tightness.
Short body hook .	Insufficient base pressure. Excessive clearance between rolls and chuck.	Can is too long—body hook being short by an equal amount. Top of seam has rounded contour.
Long body hook .	Excessive base pressure.	Always results in corresponding decrease in cover hook. "Spurs" may be formed.
Short cover hook .	Insufficient 1st operation.	Seam longer than normal—body hook normal. "Spurs" may be formed.
Long cover hook .	Excessive 1st operation.	"Cut-over" at top inside edge of seam.
Wrinkled hooks .	Insufficient 2nd operation.	Seam loose—hooks curved.
Deep countersink .	Insufficient base pressure. Excessive clearance between rolls and chuck. Chuck flange too thick.	Deep countersink is always accompanied by short cover hook.
"Skidders"—term denoting cans which slip or skid during seaming operation.	Insufficient base pressure. Worn chuck. Worn serrations on base plate. Greasy base plate or chuck. Excessive 1st or 2nd operation.	A portion of the seam is unduly wide and very loose.
"Cut-overs" .	Excessive base pressure. Excessive 1st operation. Too much clearance between rolls and chuck. Worn chuck.	"Cut-overs" are especially apparent at the overlap.
"Spurs" .	Insufficient 1st operation. Excessive 2nd operation. Too much solder at overlap. Worn rollers or roller pins. Excessive base pressure. Harsh plate.	Frequently occur at overlap. Cover hook is deficient at the site of the "spur."
Damaged can flanges.	Damage in transit, rough handling, etc.	Where flange is knocked down there may be complete failure to engage with cover hook.

APPENDIX

Temperature of Saturated Steam

Steam Pressure	Temperature	
lbs. per sq. in.	°F.	°C.
0	212·0	100·0
1	215·4	101·9
2	218·5	103·6
3	221·5	105·3
4	224·4	106·9
5	227·1	108·4
6	229·6	109·8
7	232·3	111·3
8	234·7	112·6
9	237·0	113·9
10	239·4	115·2
11	241·5	116·4
12	243·7	117·6
13	245·8	118·8
14	247·8	119·9
15	249·8	121·0
16	251·6	122·0
17	253·4	123·0
18	255·4	124·1
19	257·0	125·0
20	258·8	126·0

SIZES OF SOME COMMON ROUND OPEN-TOP CANS

Trade Name	Dimensions		Capacity	
	Diameter	Height	Fl. oz.	ml.
8 oz. . .	300	208	8·1–8·2	229–232
A1 . .	211	400	11·0–11·1	313–316
E1 . .	301	400	14·1–14·2	401–404
16 oz. . .	301	409	16·3–16·4	461–465
A1T . .	301	411	16·8–16·9	476–479
A2 . .	307	408	20·2–20·4	575–579
A2½ . .	401	411	29·7–29·9	842–848
32 oz. .	313	507	30·9–31·1	878–883
3 lb.H.R.Tall	401	711–715	50·3–52·3	1,429–1,483
A10 . .	603	700	108·5–109·2	3,078–3,098

The dimensions of cans are expressed in inches and sixteenths of an inch, thus, 411 = $4\frac{11}{16}$ inches.

These standards are those laid down in the British Standard Schedule of sizes for Tins and Cans (War Emergency), published by the British Standards Institution. The regulations governing the use of these cans, together with those for other sizes are given in the Schedule.

PRINTED IN GREAT BRITAIN BY THE WHITEFRIARS PRESS LTD.
LONDON AND TONBRIDGE